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The Influence of Infection on the Distribution of Sulfanilamide Derivatives in Blood, Cerebrospinal Fluid and Tissue

An Experimental and Clinical Study

BY

GÖSTA HERBERTS

UPPSALA 1944

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av

GÖSTA HERBERTS

MED. LIC., STOCKH.

UPPSALA 1944

ALMQVIST & WIKSELLS BOKTRYCKERI AB

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UPPSALA 1944

Translated by Carolyn King

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To my Parents

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Preface.

The present investigation has been carried out mainly at the Oto-laryngological Clinic of Akademiska Sjukhuset (University Clinic), Upsala. I should like to offer my warmest thanks to my Chief, Professor C. O. Nylén, for his great interest and valuable help while the work was in progress.

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My thanks are also greatly due to the Regnell Fund of the University of Upsala for its contribution towards expenses.

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Upsala April 1944.

Gösta Herberts.

INTRODUCTION.

The author has for some years devoted his interest to the problems presented by the treatment with sulfanilamide derivatives in oto-laryngology, with particular attention to otogenous and rhinogenous meningitis. In addition to the favourable results, the treatment of these meningitis has shown the frequent occurrence of minor toxic manifestations, as well as more serious toxic symptoms, particularly from the nervous system. This may perhaps be explained by the often unusually strong and lengthy dosage of the preparations which is necessary in certain diseases within the field of oto-laryngology. Even in a large otological clinic, however, the frequency of meningitis cases is comparatively small and the different cases vary considerably in respect of degree of illness, etiology and other factors; it has therefore not been possible to make a more detailed investigation into the therapeutic and toxic properties of the sulfanilamide derivatives on a clinical material only. The author has therefore pursued experimental studies on these problems. The main results of these investigations and their application to a small clinical material are presented in this treatise.

Part I.

HISTORICAL REVIEW.

Chapter I.

Experimental and Clinical Investigations.

Development.

At the beginning of the 20th century Ehrlich (27) and his co-workers attempted to find new remedies for treating bacterial infections. Ehrlich introduced the term "chemotherapy," defining it thus: "Was wir wollen, ist eine Chemotherapia Specifica, d. h. wir suchen Mittel, die einerseits bestimmte Parasiten zu töten im Stande sind, die aber andererseits in den zur Abtötung nötigen Mengen von dem Organismus ohne zu grossen Schaden ertragen werden." He was also successful in producing effective remedies against protozoan infections; the most renowned of these was salvarsan.

The most important advance in the work with chemotherapeutic drugs was the introduction of sulfanilamide derivatives. As early as 1919 Heidelberger and Jacobs (59) had tried to link dihydrocupreine with diazo compounds obtained from aromatic amines, including sulfanilamide to enhance the bactericidal effect. In so doing they were able to discover a good effect in *in vitro* experiments, but do not seem to have followed up their observations.

The first sulfanilamide derivative to be used clinically was *Prontosil,* which was synthesized by Mietzsch and Klarer (108), who in 1932 obtained a German patent for this drug. The first publication concerning clinical use of the preparation was Foerster's (37) communication in May 1933 about a case of staphylococcal sepsis successfully treated with prontosil. This was soon followed by a number of short clinical reports of

occasional successful treatments with prontosil. Not until Domagk's publication (24) in Deutsch. med. Wochenschrift in February 1935, was the importance of the new drug fully recognized, however. In this paper Domagk (24), who had been working with the new drug since 1932, showed *inter alia* 100 per cent healing in treatment of haemolytic streptococcal infection in mice. These results were soon confirmed by others (85, 116).

Tréfouël, Tréfouël, Nitti and Bovet (149) found in 1935 that *sulfanilamide* had the same action on streptococcal infections as prontosil; they accordingly assumed that prontosil in the organism is broken down into sulfanilamide, which would be the active factor. They were also able to demonstrate the effect of sulfanilamide on streptococci in *in vitro* experiments, whereas prontosil, according to Domagk (24), did not give an effect in such experiments. These investigations, soon confirmed by others, gave rise to an intensive research in which new preparations with a larger range of activity were sought.

Ewins and Philipps synthesized *sulfapyridine,* which was introduced in the clinic by Whitby (155) in 1938. It soon appeared that this drug had as great an effect on pneumococci as on streptococci, and fresh reports of successful treatment were published in rapid succession. In 1938 Marshall *et al.* synthesized the soluble sodium salt of sulfapyridine, after which the way was open for parenteral therapy.

A large number of new preparations were synthesized and tested experimentally and clinically. One of these drugs was *sulfathiazole,* which in 1939 was prepared independently by Fosbinder and Walter (39), Laudon and Sjögren (83), Lott and Bergeim (93), Hartman *et al.* (54). This drug has been found to have as good an effect as sulfapyridine on streptococci and pneumococci, and a better one on staphylococci, gonococci and the coli bacteria.

Experimental Investigations.

The study of the new drugs was pursued intensively everywhere, both experimentally and clinically. The experimental in-

vestigations consisted both in trying out the therapeutic effect of the drugs on bacteria in *in vitro* tests, and in testing them on experimental infections in animals. At first, the main experiment animals used were mice (34, 35, 91, 101, 103), which were infected with streptococci, pneumococci or staphylococci. Greatest interest centred round the streptococcal infection, since it was mainly against this type that the effect of the drugs first produced could be demonstrated. The experiments were usually so arranged that a series of treated animals were compared with untreated control series to discover the therapeutic effect. One after another of the newly synthesized drugs were tried out in this way, and a great number of publications appeared. Consequently larger animals, such as rabbits and dogs, were also used (50, 52, 55, 61, 74, 75, 80, 106, 111, 113, 123, 156) making it possible to study the course of different types of infection in more detail. This was particularly the case after the preparation of sulfapyridine, as interest then increased in the pneumococcal infections and their treatment. The results submitted often contradicted one another, however, and gave room for different interpretations. This may have been due in part to the fact that many of the investigators had not paid sufficient regard to the varying courses of different streptococcal infections and the varying virulence of the bacteria used; further, the blood concentration of the different drugs varied in the animals, depending on the different modes of administration.

The tested drugs were soon found to have different solubility, and a different rate of absorption and excretion within the body (34, 35, 55, 130). After Marshall (97) and his coworkers had in 1937 worked out a method for quantitative determination of the drugs in the blood and other body fluids, more thorough studies could be carried out, making possible comparisons between the different drugs. Long & Bliss (89) suggested a standardization in research in this field, on the following lines: The infection was to be induced by a certain type of bacteria, according to Lancefield (78), with a certain relatively high virulence. The elicited blood concentration of the preparation should be used for gauging the therapeutic effect. During the last few years these viewpoints have been more and

more guiding the work, and research has been conducted along considerably more exact routes (55, 103, 150, 151).

Clinical experiences.

The first published clinical results in treatment with sulfanilamide preparations were, as already mentioned, brief reports of isolated successful cases. Gradually, however, collections of more or less homogeneous materials appeared from various quarters. Greatest attention was attracted by streptococcal infections of a more septic type, such as complications in gynaecological, venerological and, not least, otolaryngological diseases. After the introduction of sulfapyridine larger series were also published of the results of treatment in pneumonias and, later on, other pneumococcal infections, above all meningitis (7, 15, 17, 19, 41, 48, 49, 60, 62, 63, 67, 72, 141, 146). The drugs were tested in a number of other diseases, such as actinomycosis (33) and in epidemic and urologic infections. Prophylactic treatments were also attempted (18). Here, too, however, the results were in many cases contradictory. Not before Marshall's (97) methods, with modifications, for determining the drug concentration had become more generally known could material, studied more in detail, be presented. After the experimental studies had laid the foundations for the evaluation of absorption, excretion, acetylation and distribution of the sulfanilamide derivatives (97), publications concerning similar investigations in the clinics appeared (87, 130, 138, 139, 158). It was in particular the Americans who worked with these experiments (87, 92, 144). Frisk (42) was making similar investigations at the same time.

Marshall (97) also found that, in the dog, sulfanilamide is excreted in unchanged form, whereas, in the other customary experiment animals and in man, it is partly excreted in a conjugated form, namely acetylsulfanilamide, which is therapeutically inactive (75). Marshall (103) also demonstrated that the conjugated substance is more toxic than the free drug. It was soon possible to establish that the other derivatives, too, are acetylated inside the organism—more exactly, in the liver (97). Thus the different facts concerning the preparations were col-

lected, and it was possible to fix certain normalized dosages. Gradually, accumulated experience has made it possible to decide approximately which drug should be given preference in the various infections.

As the investigations went on, interest came to centre round certain questions, such as the connection between the formation of antibodies and chemotherapy (26, 94), the distribution of the sulfanilamide derivatives in blood corpuscles and plasma (5, 50, 51, 52, 122, 138, 139, 140, 144, 158), and the rate of concentration in cerebrospinal fluid and tissue (1, 6, 48, 126, 127, 152). These facts had great practical importance in the study of the treatment of meningitis in particular. According to Whitby (156), Edwards (26) and Löfström (94), the chemotherapy does not affect the formation of antibodies.

Long & Bliss (89) maintain that the sulfanilamide derivatives have their perhaps most striking effect in the treatment of meningitis. Formerly, streptococcal and pneumococcal meningitis had nearly 100 per cent mortality, whereas, thanks to serum therapy and other forms of treatment, the meningococcal meningitis showed a lower mortality. When the enthusiasm following the first successful results from chemical therapy in different forms of meningitis had cooled somewhat, a more critical analysis was made of the not so few unsuccessful cases (6, 81, 82, 127). It was natural, then, that the passage of the drugs in question from blood to cerebrospinal fluid was the first thing to be studied (48, 87, 97, 126, 130, 152). The majority of investigators considered that an adequate concentration of the drugs in the cerebrospinal fluid was the most important factor in the treatment, even though voices were here and there raised for the equal importance of an adequate tissue concentration in the brain, in view of the concurrent symptoms of encephalitis (68).

The passage of different substances from the blood to the brain and the cerebrospinal fluid has been intensively studied (6, 13, 36, 45). There has been much talk about a barrier function between the blood on the one hand, and the brain and cerebrospinal fluid on the other: a blood-brain barrier and a blood-fluid barrier. The detailed function of these barriers does not

seem to have been clarified yet, so that this question will not be further touched on in this connection. The investigations made hitherto regarding the concentrations of the sulfanilamide derivatives in blood, cerebrospinal fluid and brain when the meninges are inflamed and intact respectively have been carried out without taking into account a possible barrier function.

The experimental investigations into the influence of meningitis on the passage of the sulfanilamide derivatives from blood to cerebrospinal fluid are few, while the clinical observations are abundant (6, 40, 102, 126). According to Vonkennel and Schmidt (152), sulfanilamide in healthy individuals passes from blood to cerebrospinal fluid to such an extent that the concentration of the drug in the fluid rises to 70—90 per cent of the blood concentration, whereas prontosil and uliron pass more poorly. In the clinical investigations a study has generally been made of this inter-relation between cerebrospinal fluid and blood in healthy subjects, the respective concentrations being expressed in a ratio: the cerebrospinal fluid-blood ratio or cerebrospinal fluid-plasma ratio (6, 42, 48). These values have subsequently been compared with similar values from treated cases of meningitis. Thence the interesting fact appeared that, even in healthy subjects, this ratio is very different for different preparations (42, 126). Whereas the sulfanilamide concentration in the cerebrospinal fluid is nearly the same as that in the blood (97, 152), the sulfapyridine concentration is about half, and the sulfathiazole concentration about a fifth of that in the blood (6). This applies to healthy subjects. In meningitis cases sulfapyridine shows an increase for the fluid concentration in relation to that of the blood (48), while sulfathiazole does not give the same concentration (6, 127). Notwithstanding this, sulfathiazole has in many cases given good therapeutic results in meningitis; this is probably due to its greater bactericidal properties (127).

In this connection it should be pointed out that the implications of the passage of the plasma protein to cerebrospinal fluid in meningitis have been discussed. As the preparations are thought to be bound to the plasma protein they, too, should be able to pass over to fluid in meningitis. This is not always so, however.

Several cases of meningitis have been published (6) showing increase of protein in the cerebrospinal fluid and high blood concentration of the sulfanilamide derivatives, but low fluid concentration and lethal issue.

Comparisons have also been made between the concentration of the sugar and of the sulfanilamide derivatives in the cerebrospinal fluid, of healthy subjects and meningitic cases (40). Danish observers in particular have pointed out the possibility of utilizing certain differences in the passage of the sulfanilamide derivatives to cerebrospinal fluid for a differential diagnosis of serous and tuberculous meningitis (126, 127). Hamberger (48) points out the importance of the blood-cerebrospinal fluid ratio for judging the prognosis of the individual case.

In order to get deeper into the question, a study has been made of the distribution of the preparations in the various constituents of the blood (42, 50, 51, 52, 98, 122). Frisk (42) found that those preparations which had good powers of penetrating into the cerebrospinal fluid also appeared in comparatively large quantities in the red corpuscles, whereas those found mainly in the plasma only showed low powers of penetration to the fluid.

Marshall (97, 98) showed that the concentration of *sulfanilamide* in blood corpuscles was somewhat higher than in plasma. A different distribution has been found for the other preparations. *Sulfapyridine* shows a slightly lower concentration in the corpuscles than in the plasma (42, 52); that of *sulfathiazole* is considerably lower in the former than in the latter (42, 139).

These circumstances have been studied in more detail by other investigators, who have discovered that the drug is bound to serum protein by adsorption (137). Dialysis (22) and ultrafiltration have been used. Marshall (98) reports that, in ultrafiltration, sulfanilamide has already got into the filtrate at 1.6 atmospheres. Kimmig and Weselmann (70) have shown that in ultrafiltration of serum containing sulfapyridine, the drug is retained even at 8 atmospheres. In similar tests Andersen, Møller and Simesen (1) found that most of the sulfathiazole appears in a non-ultrafiltrable form. The union with the protein is due to pure adsorption and can be wholly split by the addition of animal charcoal (70). Kimmig and Weselmann (70) have,

in cataphoretic experiments, studied to which protein fraction the preparations are bound. They found, in so doing, that the greater part of the drug went with the albumin fraction, whereas only traces could be found in the globulin fraction. Olhagen and Svartz (147) have made electrophoretic tests, showing that patients resisting salazopyrine have a high content of gamma globulin. In the favourable cases the gamma globulin and fibrinogen showed normal values, while the alpha globulin was raised.

L. Hansen (50, 51, 52) studied the distribution of sulfanilamide and sulfapyridine in blood corpuscles und plasma both in human subjects and experimentally on animals. He observed this distribution to display individual variations; he was unable to note either any sex difference, or any specific action of the infection. In a few investigated cases he found an irregular daily variation in the distribution of sulfapyridine between corpuscles and plasma while the treatment was proceeding.

Experimental toxicity.

While experiences of the beneficial effects of the sulfanilamide derivatives were being collected, an increasing number of reports of toxic manifestations were also accumulating. They were partly based on clinical experiences, but had also been studied experimentally, though in the main only on non-infected animals.

These studies followed two different lines, in that both the acute and the chronic toxicity was tested (35, 61, 92, 100, 101, 106, 110, 113, 121, 150, 151). As early an investigator as Domagk (24) had carried out *acute toxicity experiments* on mice, rabbits, and cats, and later investigators followed this lead. As the new preparations appeared they were tested as regards acute toxicity, and more and more reports collected. Nevertheless, they contradicted one another; there was no systematic testing of the drugs before Marshall (99) pointed out that the oral therapy could not be used in these experiments, suggesting instead

parenteral medication, since the crux in the evaluation of acute toxicity was to ascertain the amount of the drug absorbed. As the solubility and absorption of the different drugs varied greatly, a dose calculated for peroral administration might give a high blood concentration for one drug and a low blood concentration for another. If, on the other hand, the drug was injected parenterally and the blood concentrations determined, the comparisons between the different drugs might be more valuable. A certain standardized mode of procedure gradually emerged in this field, too. Marshall (99) determined the so-called median lethal dose, M. L. D. or L. $D._{50}$, denoting the dose from which 50 per cent of the experimental animals died within a certain number of hours; he also determined the concentration of sulfapyridine in the blood at death. It was soon possible to establish that the rabbit was more susceptible to toxic effects of sulfanilamide than the mouse, whereas the rat was less susceptible than either. According to van Dyke (150) L. $D._{50}$ in subcutaneous administration (albino mice) was, for sulfanilamide 13.5 gm/kg body weight, for sulfapyridine 3.2 gm/kg and for sulfathiazole 5.2 gm/kg. Lehr (84) frequently found death after intravenous injection of sodium sulfapyridine, due to the alkalosis of the injected drug. Symptoms of nervous irritation were noted at the same time. Kohn (74) showed that 0.5 gm sulfapyridine per kg bodyweight injected intravenously in rabbit gave convulsions leading to death in 25 per cent of the animals, while 0.8 gm/kg killed 70 per cent.

In an earlier work with chemotherapy in experimental pneumococcal meningitis in rabbits (61), the author observed severe, acute toxic symptoms to arise from the central nervous system of the *infected animals* after intravenous injection of sodium sulfapyridine, whereas the same dose and a still larger one given in the same way to healthy control animals did not cause any symptoms whatsoever. This observation was interpreted as follows: In cases of meningitis there is an increased passage of the sulfapyridine from blood to brain, achieving a toxic concentration in the brain with an intravenous dose that, in normal animals, does not release symptoms from the central nervous system.

When testing out the *chronic toxicity* the experiment animals

were put on a certain daily dosage of the drug for a fairly long period, after which they were examined in respect of weight, growth, histological changes in the organs, and mortality (8, 30, 121, 129, 151). The commonest symptoms observed for most of the preparations were of the type of *nervous disturbances,* such as ataxia, athetoid movements, opisthotonus, tonico-clonic convulsions (8, 73, 74, 106, 113, 120, 121). The histological changes in the nervous system appearing after experimental intoxication have been studied in some cases (121). Hawking (56) found a number of experiment animals to show degenerative changes, such as chromatolysis in the neurones of the anterior column of the spinal cord and in some of the nerve cells of the cortex and mid-brain. Custer (20) observed cerebral oedema, which he assumed to be the cause of the nervous manifestations; thus not attributing these latter to the direct toxic action of the drug. Other toxic symptoms observed from the nervous system were neurites, particularly after the methyl derivatives of the drug. Engelhardt and Birkenmaier (30) discovered paralytic symptoms after sulfanilamide medication in pigeons kept constantly in active and powerful motion. Other symptoms noted were disturbances in the acid-base equilibrium of the blood. Marshall (100) found acidosis in peroral medication, due to an alkali deficit; others, e. g. Lehr (84), found alkalosis after intravenous use of the strongly alkaline drugs.

Regarding the temperature, it was found that severely intoxicated animals died in a coma with a marked fall of temperature (80, 112).

The blood and the *haematopoietic system* have been studied in chronic toxicity experiments (110). Thus, Hageman (46) found haemolytic anaemia with iron pigment in the spleen. Marshall *et al.* (100) found no disturbances in the peripheral blood of rabbits and dogs after repeated large doses of sulfanilamide. The cyanosis observed could not be explained experimentally.

The *excretory organs* have been much investigated in connection with sulfanilamide medication. A number of cases showed calculi (3) which, after the use of certain drugs, tended to spread enormously so as to stop up the renal pelvis and the ureters. Molitor (111) observed a case of this kind, where the

calculi had been set up by acetylsulfapyridine. Toxic lesions to the renal tissue (3, 151) constituted another type of disturbance.

Lesions to the liver have not been noted. Nitti and Bovet (116) found that guinea-pigs sensitized intradermally with prontosil showed skin reactions after prontosil medication. This does not apply to sulfanilamide.

Toxic symptoms in man.

After commencing the treatment with sulfanilamide derivatives in human subjects it was not long, as already mentioned, before toxic manifestations were reported, in the form both of general symptoms and of disturbances localized at certain organs. The manifestations from the *nervous system* were the first toxic symptoms observed as a reaction of the treatment, and they are considered to be the commonest signs of intoxication (79, 105, 157). Their genesis has been discussed, as it proved difficult to decide whether the symptoms in question were released by the disease itself or were due to the therapy (38, 125). Bruun (14) and others maintain a combination of toxic lesion from the infection and the drug. It is therefore difficult to say what frequency the toxic effect of the sulfanilamide derivatives has in these respects. In certain cases, however, so direct an action of the drug has been observed that it seems legitimate to assume a direct toxic action from it.

According to Lassen (82) and Marshall (100) the symptoms from the central nervous system are to be regarded as purely toxic action of the drug, whereas a number of other symptoms commonly occurring are due to allergy.

Dizziness, which often appears, sets in more frequently after administration of sulfanilamide than efter treatment with sulfapyridine and sulfathiazole (90). Long & Bliss (89) have pointed out that alcohol enhances the dizziness. Headache, which is also common, may be a warning sign of the approach of more serious complications (89, 157). Other symptoms commonly appearing are depression, somnolence, marked malaise, nausea and vomiting (43, 90). The last two symptoms have, as a rule,

been taken as indicating a central toxic effect, especially as they appear in parenteral therapy also (9, 105). If they set in soon after peroral medication, they are supposed to be due to a local irritation.

Slight mental derangements are not uncommon. Thus, depression, difficulty in concentrating on any one task, and mild states of confusion are usual (71, 89). Disorientation and hallucinations have been observed in a number of cases. Maniac states and other psychoses are rare, but have been described (4, 43).

The appearance of *local symptoms* due to a toxic effect of the drug has been studied by a number of investigators. The figures they submit *re* the frequency vary extremely, however. This may in part be due to difference in dosage. In these investigations no study has been made of the relation between the blood concentration of the drugs and the toxic manifestations observed. The frequency figures submitted in the following pages in this chapter, have been taken, when no other source is given, from the publication by Long & Bliss (90) on the toxicity frequency shown by a material of cases where 1000 were treated with sulfanilamide, 297 with sulfapyridine and 271 with sulfathiazole.

Neuritis.

With regard to toxic symptoms set up by sulfanilamide derivatives in the nervous system, a large number of case reports have been published of neuritis after medication with the drug (14, 28, 133). Although these symptoms have been described as appearing after most of the preparations used, the vast majority set in after methyl derivatives. Cases of amblyopia (2) and encephalopathy (2, 38) after a normal dose have been described. Nyman (117) has pointed out that the methylation may have a part in the enhanced toxic effect on the brain, since an effect of this kind appears even after methylation of the ordinary alkaloids such as strychnine, morphine, cocaine, quinine.

The neurites are commonly of motoric type, although disturbances of the sensitive nerves have also been described (14, 133). Initial signs observed are paraesthesias and neuralgic pains in arms and legs. In most cases the symptoms disappear after the discontinuation of the drug, but sometimes a severe

paralysis of the legs may persist (28). One case of death has been reported which at the autopsy showed softening of the spinal cord (136). The neurites do not as a rule appear until about 3 weeks after finishing the treatment (133), and it has been observed both clinically and experimentally that they are increased by muscular exertion (14, 30). The possibility of vitamin B deficiency as causative factor has also been discussed (89).

Cyanosis.

Cyanosis is a toxic symptom often noted in treatment with the sulfanilamide derivatives. It is particularly pronounced and frequent in medication with sulfanilamide, but it is not considered an alarming symptom calling for the discontinuation of the drug (90). A number of investigations into its cause have been published. In the beginning cyanosis was attributed to the formation of sulfhaemoglobin, but this proved not sufficiently constant to explain all cases. Kallner (69), has found the cyanosis due to combination of the amide component in sulfanilamide with a special form of haemoglobin, namely, the so-called carbhaemoglobin.

Disturbances in the acid-base equilibrium.

The appearance of an acidosis during the treatment with sulfanilamide was observed at an early date (89). The frequency is given as 1.9 per cent. Southworth (143) found in clinical cases a decrease in the plasma's carbon dioxide combining power. He subsequently found that if this decrease was present in a marked form a hyperventilation arose, and a non-compensated acidosis was set up. These observations were later on confirmed by other investigators. The concurrent use of alkali carbonates or citrates with sulfanilamide therapy was therefore recommended, especially in the American literature. The more extensive use of the soluble alkaline salts of the preparations altered the question somewhat: these preparations are highly alkaline, (p^H about 10—10.5), and certain investigators found alkalosis following parenteral therapy (84).

Fever.

The first to describe fever during treatment with sulfanilamide derivatives were Long & Bliss (89). The term "drug fever" was introduced by Hageman and Blake (47), who studied these matters more closely. Drug fever is observed with particular frequency in association with exanthema, but it may also appear as an isolated symptom, and is considered to be a warning sign of more serious complications (89, 128). Following the onset of the fever the patient begins to feel unwell; headache and impaired general condition set in. The fever appears on the 5th—9th day after commencing the therapy. According to the American literature (90) 10 per cent of the treated cases get fever after sulfanilamide, 4 per cent after sulfapyridine, and 10 per cent after sulfathiazole. According to Bäfverstedt (16) fever in sulfanilamide treatment is more common among males (9 per cent) than among females (3 per cent). His material consisted of 236 cases: 151 males and 85 females.

These figures apply only to adults, as fever is considerably rarer among children. Nissen (114) points out that the fever presents certain similarities with serum sickness. Hageman and Blake (47), Mellon, Gross and Cooper (107) consider the fever to be due to haptene action, in that a union of the drug, or modified form of it, with native protein acts as an antigen. "Drug fever" is considered to be a serious toxic symptom, demanding the immediate cessation of the medication (90). Different types of febrile reaction have been seen, in the form either of a continued fever or high temperature peak, or of remittent fever. It has been frequently pointed out how difficult it is to judge whether the fever is due to the disease as such, or whether it is due to a toxic action from the drug; Nissen (114) maintains, however, that the late onset of the fever at a stage when successful chemotherapy has produced afebrility makes the latter alternative the more probable, provided other complications can be ruled out. "Drug fever" is specific to the preparation inducing it and does not prevent continued therapy with another sulfanilamide derivative (90, 114).

Dermatitis.

Toxic manifestations from the skin in the form of rashes have been observed after most of the drugs used (82, 90, 157). These symptoms appear to 1.9 per cent after sulfanilamide, 2 per cent after sulfapyridine and 5 per cent after sulfathiazole therapy. As has already been said, they often accompany "drug fever," although they may also set in appreciably earlier in the course of the treatment. According to Bäfverstedt (16) exanthema in sulfanilamide treatment is considerably more usual in women (18 per cent) than in men (5 per cent). It usually sets in after a high dosage over a fairly long period, but it has been pointed out by certain authors (43) that there is no direct relation between the blood concentration and the onset of exanthema; this symptom has even been observed after one single dose of moderate size (4). Aubert (4) has demonstrated exanthema in a blood concentration of 2.4 mgm. per 100 ml. Attention has been called to the fact that the patient who has once reacted with exanthema will be very liable to it in later medication also (114). To obviate this it has been suggested that such patients undergo skin tests. A large number of different forms of toxic exanthema have been noticed: scarlantiniform and morbilliform types are particularly common, as also efflorescences resembling erythema nodosum. Isolated cases of severe exfoliative dermatites have also been described (16). Exanthema is not as a rule looked upon as a serious toxic manifestation, as it usually subsides rapidly after the drug has been discontinued (90). A number of authors have, in serious cases, continued the therapy in spite of the onset of exanthema, without any grave consequences accruing. According to Lassen (82) and Marshall (100), not only fever but also exanthema are to be taken as allergic symptoms, to be explained in the way mentioned above, i. e. a haptene action.

Kidney lesions.

Among the early observed toxic manifestations of the sulfanilamide derivatives were the kidney lesions (98). Two different types can be distinguished, one of which is due to precipitations of the acetyl derivatives in the urinary tract, followed by haematuria and sometimes anuria. A number of observations

have been made of calculi in the urinary tract resulting from sulfapyridine and sulfathiazole medication (90). Analysis has, as already mentioned, shown these to be formed by acetyl derivatives of the drugs. Svartz (147) considers an acetyl hydrochloride to be the offender here. The calculi observed range from small ones in the renal tissue to large ones filling up the renal pelvis and ureters (90).

Another form of action observed was a toxic lesion to the renal parenchyma, as in mercury poisoning, mainly localized at the tubuli. There was concomitant rise of non-protein nitrogen and hypochloraemic uraemia (12, 159).

These lesions have not been observed after sulfanilamide medication. The frequency of haematuria following sulfapyridine medication is 8 per cent and, following sulfathiazole medication, 2.5 per cent, while corresponding figures for anuria are 0.3 per cent and 0.7 per cent respectively.

Liver disturbances.

Liver disturbances have been observed less frequently during treatment with sulfanilamide derivatives. Frequency figures have not been given. There are descriptions of isolated cases where such derangements appeared, but they had in the main a good prognosis. De Bonis (23) has carried out liver-function tests before and after sulfanilamide therapy. In so doing he was able to establish a lowered function in 5 of the 11 cases, and assumed that a derangement of the liver function was particularly usual in association with this therapy. Another author, Schmidt (132), who carried out similar investigations, came to the opposite result, inasmuch as none of his cases showed any functional disorder after the treatment. The question is therefore open, even if clinical experience argues for the infrequency of serious liver disturbances during this treatment.

Toxic action on the blood and the haematopoietic system.

The gravest toxic effects from the drug have been observed in the blood and the haematopoietic system (89). Quite early on, a not infreqent (3 per cent), moderate haemolytic anaemia was observed (119), while the cases of severe acute haemolytic

anaemia occur in 1.8 per cent. Both these forms of haemolytic anaemia have been observed mainly after therapy with sulfanilamide.

The toxic effect on the white corpuscles has been the subject of great interest. It was soon found that moderate leucopenia often resulted from the treatment, but more serious harm was also observed, extending as far as to agranulocytosis (82, 90, 114). While mild leucopenia sets in early during the treatment, agranulocytosis does not appear until the third week after starting the treatment and, in many cases, after it has been discontinued (90). The prognosis of this agranulocytosis is poor in that two thirds of the cases hitherto published died, despite all treatment (82, 114). This form of agranulocytosis has therefore been considered to be a specific action of the sulfanilamide derivatives. It is commonest after sulfapyridine, 0.3 per cent; it appears after sulfanilamide to 0.1 per cent, and is hardly noticeable after sulfathiazole (90). In contrast to this it has been found that an agranulocytosis of another genesis does not contraindicate the use of the drug. Marshall (100) and Lassen (82) consider that agranulocytosis, too, is to be regarded as an anaphylactic reaction to the drug.

Another condition observed during treatment with the drug is leucocytosis (90). This also has been taken to be an anaphylactic symptom, as it was noted in association with other reactions of hypersensitivity to the drugs.

Other lesions.

Isolated observations of toxic symptoms from other organs have been published (65, 66, 109, 123). A number of cases of death following intravenous prontosil therapy have been described (31, 142).

Complications in local treatment.

Of great interest are the local symptoms observed in association with chemotherapy administered locally in meningitis and processes in the vicinity of the brain. Most of these symptoms have appeared after intralumbar administration of the soluble

sodium compounds of the derivatives (29). Autopsies have revealed necrosis of the spinal cord and roots, attributed to the alkalinity of the drugs used. Watt and Alexander (154), Koch (73), Pilcher, Angelucci and Meacham (120) have found that local use of sulfathiazole in infectious processes in or near the brain, particularly the motoric region, has caused epileptiform fits which in a number of cases led to death in status epilepticus. A warning is therefore issued against the use of these drugs locally on traumatic lesions within this area. Experimental studies of these complications have been published by Botterell (11) and Holmes (66), Watt and Alexander (154) and Koch (73).

Chapter 2.

Short summary of the literature leading to author's problems.

Questions bearing on sulfanilamide therapy have been the object of lively scientific interest. The therapeutic effect of the different preparations have been studied, and the acute and chronic toxicity tested on healthy animals. Little study has, however, been devoted to the toxic effect of the drug on infected animals, and investigations into the distribution of the drugs within the organism of infected and healthy animals respectively have not been carried out on sufficiently large series. Nor has the clinical research been able to elucidate either the influence of infection on the distribution of the drugs within the organism or the significance of infection for the onset of the toxic manifestations. These circumstances may partially explain the often conflicting statements in the literature.

The clinical and experimental observations suggest, as has been mentioned before, that mild toxic symptoms from the central nervous system are the commonest toxic manifestations of the drug. More serious toxic symptoms with epilepsy, psychosis, and so on, are not unduly rare specially after local treatment in or near the brain. The concentration of the drug in the brain of

infected and healthy animals therefore seems to merit a closer experimental study.

The passage of sulfapyridine from blood to cerebrospinal fluid has been proved, both clinically and experimentally, to be greater in the presence of meningitis than in health. Frisk (42) has found a connection between the passage of the drugs to cerebrospinal fluid and the distribution in blood corpuscles and plasma in healthy human subjects; these questions, too, should therefore be studied in healthy and infected animals.

The questions to be solved at present seem to me, therefore, to be the following: Does an experimental infection with meningitis in rabbits make for any difference in the distribution of sulfapyridine within the organism in relation to the same distribution in healthy rabbits? Does another type of infection, e. g. sepsis, affect the above-mentioned circumstances in any way? Can an increase of the toxic manifestations released from the brain be explained by a possible change in the distribution of sulfapyridine within the organism in the presence of infection? In man does infection make for any difference in the distribution of sulfanilamide derivatives in corpuscles and plasma as compared with the same distribution in the healthy subject? And if this be the case how can the action of infection be explained?

Part II.

MY OWN INVESTIGATIONS.

EXPERIMENTAL SECTION.

Chapter 3.

Material and Methods Used.

Material.

Male white rabbits from the same breed were used in the experiments, except in one series for investigating the sex distribution. The weight of the animals was about 2.0 kg, with variation from 1.6—2.4 kg. The diet was uniform. Sedimentation rate was taken on healthy animals as control. Temperature controls were made on animals investigated in series.

General Course of the Investigation.

The concentration of sulfapyridine in blood, plasma, cerebrospinal fluid and tissue in rabbit was determined, after the drug had been injected in the form of the sodium salt by the intravenous, intramuscular or subcutaneous route. To obtain general guiding-lines, these investigations were first made on a few animals, where the concentration in blood, plasma and cerebrospinal fluid was followed for 3—5 hours after injection with the drug. Certain characteristic curves were obtained, and then the actual investigation was carried out on series of healthy and infected animals. In a smaller series the concentration of sulfapyridine in the blood, plasma and fluid was observed in the same animal before, during and after the experimental infection.

Methods.

The injection of sulfapyridine.—The preparation used was Septipulmon, hereafter called Sp., which is a 20 per cent alkaline solution of sulfapyridine. Its p^H is 10.2. The sulfapyridine was injected into an ear vein in a quantity varying from 0.10—0.20 gm per kg bodyweight. The injection was made slowly during a time of 1 ½—2 minutes. When administered intramuscularly, the drug was injected into the thick musculature of the hind legs in a dose of 0.3 gm/kg. When used subcutaneously the drug was injected under the dorsal skin fairly far back. Dose: 0.4 gm/kg.

These doses were chosen to get more or less comparable concentration values in the blood at the time for sample-taking.

Sample-taking.

Blood.—5—6 ml of blood are taken from an ear-vein not used for injection with a syringe rinsed through with heparin.

Cerebrospinal fluid.—Cerebrospinal fluid is obtained by a cistern puncture in the usual manner. The fluid runs out in rapid drops. A tuberculin syringe is applied, after which 0.6 ml of fluid is very carefully removed. No anaesthetic is necessary. To begin with, the author worked with animals under narcosis, but found the puncture more difficult to perform and the risk of injury greater. The fact that the animal goes taut during the operation makes the fluid run more quickly. After a successful puncture the animal shows no symptoms whatever.

Tissue.—The animal is given ether to produce moderately deep narcosis. The thorax is then cut up and the tip of the heart snipped off, whereupon the animal rapidly bleeds to death. Small pieces are taken from the organs wanted and laid on a porcelain dish. Care is taken not to soil the organs with blood or let them touch one another. The organs must not be allowed to dry from exposure to air.

Bacteriological Methods.

For infecting the animals a strain (S. B. L. 78) of pneumococcus type 1 has been used, obtained from the State Bacterio-

logical Laboratory. The strain has maximal virulence for mice —i. e. 1—5 bacteria kill mice in the course of 4 days.

The strain has been kept dried in evacuated glass tubes. It was left to grow in ascitic bouillon, to which about 0.5 per cent glucose is added after 8—10 hours. After a further 2 hours in a thermostat at 37° C, 0.3 ml of the culture is injected intraperitoneally into mice. Cardiac puncture is performed on the dead mouse. Culture from the cardiac blood is made in the same medium as above. The culture is fully grown in about 8—10 hours, after which it is tested for purity, capsular swelling and agglutination in the following manner (95).

Capsular swelling according to Neufeld: A normal loopful of the culture is placed on a slide and mixed with a loopful of pneumococcus type 1 serum, after which a loopful of Löffler's alkaline methylene blue diluted 1 : 2 is added for staining. A cover glass is put on and the preparation is ready for observation under the microscope. Two phenomena are looked for in the microscope, capsular swelling and agglutination.

The culture is diluted with physiological saline solution, after which it is ready for injection.

Infection of the animals.

M e n i n g i t i s.—Dilution 1 : 100 of the culture. 0.3 ml is injected subarachnoidally after cistern puncture in the scruff. To prove that the needle has got into the cistern and has not gone too deep it is necessary to control, firstly, that fluid runs out of the needle, secondly, that there is no resistance at injection and, thirdly, that the animal reacts with nystagmus in one direction or the other immediately after the injection: this lasts only $^1/_2$—1 minute (caloric reaction—room temperature of the culture). Otherwise the animals show no reaction. Meningitis is controlled by cultures made when the fluid samples are taken, and by sedimentation rates.

S e p s i s. — An intradermal injection of 0.2 ml of the culture diluted 1 : 10 is made on a shaved area of the dorsal skin, according to Goodner (44). Control by blood cultures and sedimentation rates.

Vaccination.

For this process a vaccine of the same strain is used, obtained from the State Bacteriological Laboratory. The vaccine con-

sists of bacteria killed with 2 per cent formalin in physiological saline solution. The bacterial concentration is about 1000 million bacteria per ml. The vaccine is injected intravenously following Löfström's mode of procedure, i. e. 1st day: 0.2 ml, 5th day: 0.4 ml. On the 8th day the agglutination titer is determined (95).

Determination of agglutination titer.

Serum dilutions from the vaccinated animals of 1 : 2, 1 : 4, 1 : 8, 1 : 16, 1 : 32 and 1 : 64 are put into test tubes. To each of these is added an equal quantity of the vaccine diluted 1 : 25. The tubes are placed 1 hour in a thermostat at 50° C, and then left in a refrigerator over night. The agglutination is read off macroscopically.

Determination of Sulfanilamide Derivatives in Body Fluids.

For this determination Frisk's modification of Marshall's method has been used (42). The method is based on the presence of a free primary amino group in the para-position. The amino group is diazotized and the diazo compound coupled with an aromatic amine, producing an azo dye which is measured colorimetrically.

Solutions required.

1) A solution of trichloracetic acid containing 15 gm dissolved in water and diluted to 100 ml.
2) An aqueous solution of sodium nitrite containing 1 gm per 100 ml.
3) An aqueous solution of sulfamic acid, containing 0.2 gm per 100 ml.
4) An aqueous solution of ethyl-α-naphthylamine hydrochloride containing 0.3 gm per 100 ml. This solution should be kept in a dark bottle.
5) An aqueous solution of saponin containing 0.5 gm per litre.
6) A four times normal solution of hydrochloric acid.
7) Methanol.

Determination in the blood.

Free sulfanilamide derivative.—To 2 ml of heparinized blood are added 30 ml of saponin solution. After two or three minutes the content is precipitated with 8 ml of the trichloracetic acid solution, and the mixture filtered. Two ml of the filtrate is placed in a test tube graduated at 10 ml and 0.1 ml of the solution of sodium nitrite

is added. After one or two minutes, 1 ml of the sulfamic acid solution is added and the ingredients are mixed thoroughly. After another two minutes 1 ml of the ethyl-α-naphthylamine solution is added. The mixture is then made up to the 10 ml mark with methanol, shaken and allowed to stand for at least ten minutes. The red colour is measured in a Pulfrich "Stufenfotometer," with a microcell, a layer of 50 mm and a S 53 filter. Methanol was used as a comparative fluid.

Total sulfanilamide derivative.—To 20 ml of the blood filtrate is added 1 ml of the solution of hydrochloric acid. The mixture is boiled in a reflux condenser for thirty minutes. It is then cooled and the volume adjusted to 20 ml. Two ml of this solution are taken, and the method continued as described above.

The concentration of drug in the blood was determined from the extinction value by means of standard blood curves.

Determination in blood-plasma and cerebrospinal fluid.

The concentration of the different sulfanilamide derivatives in the plasma and cerebrospinal fluid was estimated the same way as in the blood. The concentrations were determined from standard-plasma and standard-water curves respectively.

In those cases where repeated sample-taking of blood and cerebrospinal fluid did not allow sufficient quantities to be extracted, 1 ml was used for the blood determination and 0.5 ml for that of the cerebrospinal fluid. Corresponding amounts of the reagents were used in so doing, so that the filtrate had the dilution 1 : 20. The same method as above was then followed, with the exception that the amount available for hydrolysis was smaller.

This modification has been tested and employed by several authors (42, 49, 114, 123), who found it satisfactory. Repeated 0 tests have been made on the fluids for a control of the method.

The standard deviation of the method.

The standard deviation of the method was calculated from 52 double determinations on 52 samples of blood from rabbits and patients undergoing treatment with sulfanilamide derivatives. Both the free and the total compound were estimated.

The formula $\sigma = \pm \sqrt{\frac{\Sigma d^2}{2N}}$ was used (21). The standard deviation was 0.60 ± 0.059 mg for the free substance and 0.68 ± 0.067 mg for the total substance. This makes 2.59 per

cent and 2.67 per cent for free and total compound respectively calculated on the averages for all the double determinations.

Determination of Sp. in Tissue.

The following modification of Richtner's (123) method has been used to determine Sp. in tissue. The procedure is, briefly, this: The weighed tissue fragments are boiled to pieces in sodium hydroxide, which is neutralized with hydrochloric acid after dilution with distilled water and precipitated with trichloracetic acid in quantity to give the dilution 1 : 20. The filtrate obtained is treated in the same way as was described for blood.

In detail the procedure was as follows:

0.2 gm of moist, finely divided tissue is put into a centrifugal tube with 1 ml of 5 per cent sodium hydroxide and boiled 1 1/2 minutes in a water-bath, stirred all the time. After cooling, 1.6 ml of distilled water is added, after which neutralization is done with 0.4 ml of four times normal solution of hydrochloric acid. After stirring the content is precipitated with 0.8 ml of 15 per cent trichloracetic acid. Renewed stirring with a glass rod, after which the mixture is left to stand 15 minutes. Centrifuging for 10 minutes, 3000 revolutions per minute. 2 ml of the clear supernatant fluid is pipetted off and transferred to a test tube graduated at 2 and 10 ml respectively. 0.1 ml of four times normal solution of hydrochloric acid is added. Boiling in a water-bath for 30 minutes. Filling up with distilled water to the 2 ml line when required. After cooling, follows diazotization, coupling with ethyl-α-naphthylamine and dilution with methanol to the 10 ml line, in the same way as was previously described for blood. The extinction values are read off on the standard curve, which was made after addition of Sp. to the tissue.

Validity of the method.

The method has been found extremely satisfactory. It calls for a small amount of tissue only, and can suitably be used in large serial investigations, which in this case was necessary. Boiling the tissue to pieces in sodium hydroxide naturally means that part of the acetylated Sp. is transferred in a free form, in spite of the short time of 1 1/2 minutes. The method can therefore not be used for determining free Sp. in tissue. In this investigation, however, the intention is first of all to study the toxic effect of the drug in the tissue. Acetylated Sp. is more toxic than the free drug, as mentioned before. Richtnér (123)

has tested how much of the acetylated derivative is transferred in a free form during this boiling in sodium hydroxide, and has found a value of about 70 per cent. In similar experiments the present author found values varying from 60—72 per cent.

Boiling with sodium hydroxide.—The submitted time of 1 1/2 minutes is, in most cases, sufficient to disintegrate the organs completely. If this is not so, they are given a further 1/2 minute in the water-bath, stirred all the time. To discover how this effects the values the following test has been made: In 3 different tests a boiling time of 3/4 minute, 1 1/2 minutes and 4 1/2 minutes respectively is used. The figures obtained were:

0.70	0.70	0.70
2.30	2.25	2.40
19.00	19.20	19.00

The variation in the figures does not exceed the method error, so that prolonged boiling has no significance.

Neutralization.—At neutralization an abundance of chloride ions are obtained in solution. In earlier methods of blood determination, according to Marshall, this was observed to have a disturbing effect in the coupling with ethyl-α-naphthylamine. After Marshall (99) had introduced his modification with the removal of the nitrite surplus with sulfamic acid, he reported that a disturbing effect was no longer to be seen. The sodium chloride content of the mixture is less than 1 per cent, which cannot cause precipitation of linked dye. Nor has precipitation of the fluid been observed.

Dilution with distilled water and precipitation with trichloracetic acid.—To prevent too much Sp. accompanying the subsequent precipitation with trichloracetic acid, a dilution is required which in ordinary blood determination must not be lower than 1 : 20 (99). In this case, as in blood determination, the dilution of the mixture will be at least 1 : 20. The possible loss from part of the Sp. accompanying the precipitation is eliminated by reading off the extinction values, as described above, on the standard curve of the tissue.

The same conditions hold for the subsequent course of the method as for determination in fluids, so that no further study of these factors is indicated.

Testing of the method.

The following tests have been made to control the method:

1) 0 test of the tissue.—Tests made on normal tissue not containing Sp. have given negative results.

2) Addition tests.—Addition tests have been made to discover whether any of the tissues included in the investigation shows a difference in relation to the rest, with regard to the Sp. concentration obtained after addition of equal quantities of Sp.

This has been done as follows:

To 2 gm of fresh moist tissue is added Sp. solution in an amount to give the mixture a concentration of 6.7 mgm sulfapyridine per 100 gm tissue. The finely divided tissue is carefully mixed with the added Sp. and left in the refrigerator over night. The next day 5 double determinations are made on each organ. These determinations give the following mean values:

Brain	6.58	mgm	per	100	gm
Liver	6.63	»	»	»	»
Spleen	6.68	»	»	»	»
Lung	6.82	»	»	»	»
Muscle	6.62	»	»	»	»
Blood	6.58	»	»	»	»

As a further control of the method, double determinations were made on blood samples in 24 cases, according both to the ordinary blood method and to the tissue method. The following values were obtained:

Tissue method	*Ordinary blood method*	*Difference per cent*
5.4	5.6	+ 4 %
8.5	8.2	— 4 »
24.0	23.3	— 3 »
33.7	34.2	+ 1 »
10.7	11.5	+ 7 »
22.9	23.2	+ 1 »
32.7	31.0	— 5 »
19.0	19.6	+ 3 »
19.6	19.6	0 »
17.6	18.0	+ 2 »
16.8	18.0	+ 7 »
21.3	20.7	— 3 »
19.0	18.8	1 »
18.5	19.1	+ 3 »
18.5	18.2	— 2 »
10.1	9.8	— 3 »
10.7	10.0	— 7 »
11.8	11.3	— 4 »
11.1	11.7	+ 5 »

Tissue method	*Ordinary blood method*	*Difference per cent*
21.3	20.7	— 3 %
20.7	20.2	— 2 »
22.9	23.8	+ 4 »
16.3	17.2	+ 6 »
27.6	28.3	+ 3 »

The good agreement between the figures postulates that the micro-method for tissue determination must be considered satisfactory.

Standard deviation of the method.

The standard deviation of the method was calculated from 150 double determinations on 150 pieces of tissue from rabbits undergoing treatment with sulfanilamide derivatives. Only the total Sp. was estimated. The standard deviation was 0.50 ± 0.029 mg. This makes 3.91 per cent of the average for all the double determinations.

Chapter 4.

Course of Infection and Histological Studies.

Course of Infection.

Animals Infected Subarachnoidally.

After a few preliminary trials in order to find the most suitable dilution of the bacteria culture, a first series of animals was infected according to the method prescribed; a study was then made of the course and fatality of untreated meningitis. This series comprised 15 animals. The infected animals were controlled by cultures on blood and spinal fluid at regular intervals after the onset of infection. Blood cultures, carried out on 6 of the animals 3 hours after the bacterial injection, were positive in 4 cases. The same animals had negative blood culture 9 hours later. Samples of cerebrospinal fluid were taken 3, 6 and 12 hours respectively after the bacterial injection, each time from 2 animals not hitherto used. Cultures from all these samples were positive. The 12-hour samples showed a slight opalescence in the fluid, an abundance of pnemococci type 1 in the smear,

and a moderate cellular increase. During the first 24 hours the animals presented extremely mild symptoms. The animals ate, drank and moved about as usual. After 24 and 48 hours the blood cultures were negative in all the animals; fluid samples now taken for the first time from 3 further animals showed increased findings and positive cultures. During the second day the animals were observed to move about less; most of them neither ate nor drank. Towards the end of the second 24 hours and beginning of the third day most of the animals showed an incipient weakness in the hindlegs. Six of them died during this stage showing symptoms of increasing paresis and respiratory paralysis. The remaining animals died from similar symptoms during the third day.

As the course of the disease of the infected animals showed individual variations—some animals succumbing earlier than others—the following therapy was applied with regard not to the point in time after the onset of infection but to the symptoms observed. As a rule the animals became markedly affected 48—70 hours after the bacterial injection; the therapy was therefore applied within this interval. At this juncture the animals were showing signs of *peripheral circulatory collapse* (cold ears, collapse temperature in the animals then investigated, difficulty in obtaining blood from the ear-vein, small amount of blood yielded from tapping at death). Blood and cerebrospinal fluid cultures were made in connection with sample-taking, for determining the concentration of Sp. in the treated animals. The spinal liquor cultures were positive in all cases, and the blood cultures positive in 90 per cent.[1]

Vaccinated Animals, Infected Subarachnoidally.

It appears from the above, as has also been pointed out in an earlier paper (61), that experimental pneumococcal meningitis has a very rapid course in the experiment animals. A more gradual course can be obtained by first vaccinating the animals with specific pneumococcus vaccine, after which they can be

[1] As both the 12-hour, 24-hour and 48-hour blood samples showed negative cultures, these positive cultures in the blood at the time for sample-taking must be regarded as terminal phenomena. As mentioned above, the animals 3 hours after the bacterial injection showed a temporary bacteriaemia, too.

infected subarachnoidally with a considerably larger dose. The course of such a meningitis will not be so acute and rapid; the animals live longer and show more pronounced symptoms. The following test was made to study the effect of Sp. on a protracted and advanced meningitis of this kind: A series of animals were vaccinated according to the method prescribed, and the agglutination titer determined in the 9th day. It proved very easy to obtain a low agglutination titer in this way. The titer values are shown in tables 14 and 15. On the 9th day after the first injection of vaccine these animals were infected subarachnoidally with 0.3 ml undiluted culture, i. e. a 100 times larger dose than that in non-vaccinated animals. The course of the meningitis thus induced was considerably slowed up. In a number of cases the symptoms were so slight that the animals had to be re-infected with the same dose after 48 hours. It took 4—5 days from the first bacterial injection for the animals to reach the same degree of illness as the non-vaccinated animals showed after 48—70 hours. Cultures from the cerebrospinal fluid samples taken for determining the concentration of Sp. were positive in all cases, whereas the blood cultures were negative in all cases.

Animals Infected Intracutaneously.

The animals were infected intracutaneously according to the method prescribed (44) to induce sepsis. A sharp local reaction with redness, swelling and eczematous changes in the area round the infected part could be observed on the 2nd day. The blood cultures were in most cases positive 48 hours after the bacterial injection; in the few cases where it was negative the animals were re-infected at this juncture. Not until the 3rd—4th day after infection was it possible to observe more pronounced symptoms in the animals in the form of lowered mobility and loss of weight. The animals did not eat or drink. On the commencement of the therapy, then, the blood culture was always positive. Cultures on spinal fluid, taken simultaneously, were negative in all animals except for 1 case.

In the following pages the animals infected by a cistern puncture in the scruff will be designated "meningitic animals," and those similarly infected after first having been vaccinated,

"vaccinated meningitic animals." The animals infected intracutaneously will be called "septic animals."

Histological Examination.

To investigate the histological picture of the meningitis in different stages a smaller series of untreated animals was infected according to the methods prescribed. The animals were killed at different intervals after infection, and their brains examined histologically. Brains of treated meningitic and septic animals were likewise examined histologically. In addition, attempts were made to discover the localization of Sp. in the cerebral tissue.

Histological technique.—To study the development of the meningitis and the effect of the treatment, the brains of the killed animals were fixed immediately after removal in 95 per cent alcohol. After embedding in paraffin the sections were stained with Haematoxylin—van Gieson. The microscopic examination of the sections showed the following:

Untreated meningitic animals.—This series showed a continuous progression of a purulent leptomeningitis localized both at the base and the convexity. In the advanced stages the inflammation was also observed to penetrate into the superficial layers of the brain substance.

4-hour meningitis.—A slight, oedematous thickening of the soft membranes with swelling of the arachnoidal cells, hyperaemia and sparse emergence of polynuclear leukocytes in the immediate surroundings of the vessels (fig. 1).

3-day meningitis.—Purulent leptomeningitis of great intensity, marked disintegration of cell-nuclei and in places necrosis in the exudate (fig. 2).

Sp.-treated meningitic animals.—The treated series shows a microscopic difference from the conditions in the untreated specimens only in the cases where the treatment had been going on longest.

2 1/2-day (60 hours) meningitis.—(Sp. injected 18 hours previously.) Moderately pronounced, purulent leptomeningitis, in many places with disintegration of cell-nuclei and necrosis of the macrophages (fig. 3).

3 3/4-day (90 hours) meningitis.—(Sp. injected 18 hours previously.) Intense, purulent leptomeningitis with haemorrhages, leucocyte infiltration of the superficial stratum of the cortex and continuation of the meningitis along the vessels into the cortex. At several places there is a peculiar endovascular proliferation of the endothelium in the pia vessels, whereby the lumen seems more or less filled by a reticular or syncytial cellular tissue. Endovasculitic change of this

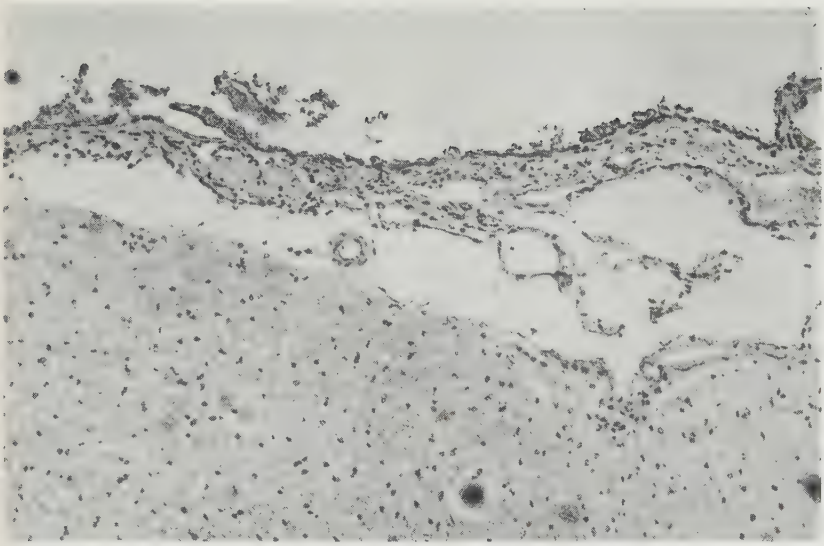

Fig. 1. Rabbit No. I. Four hours after subarachnoid injection of pneumococci. No treatment. Initial leptomeningitic change, visible as arachnoid epithelial desquamation, hyperaemia and slight increase of meningeal macrophages.

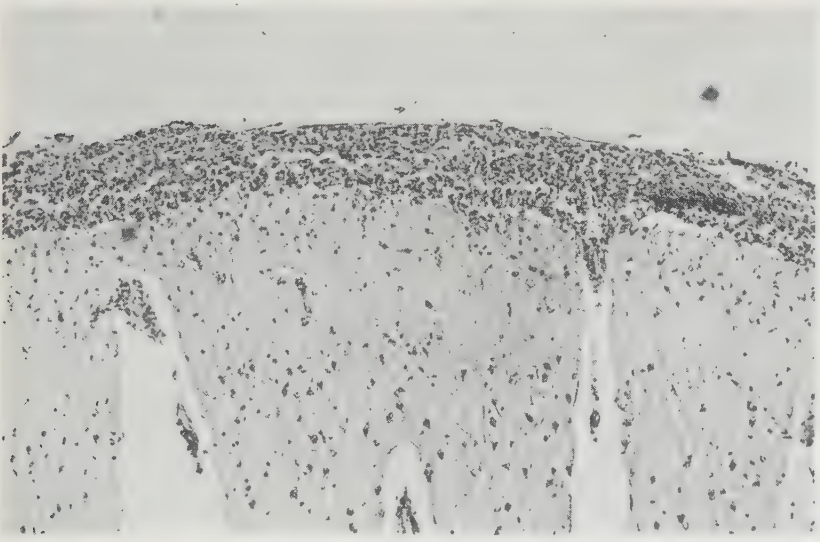

Fig. 2. Rabbit No. III. Three days after infection. No treatment. Purulent leptomeningitis in severe development.

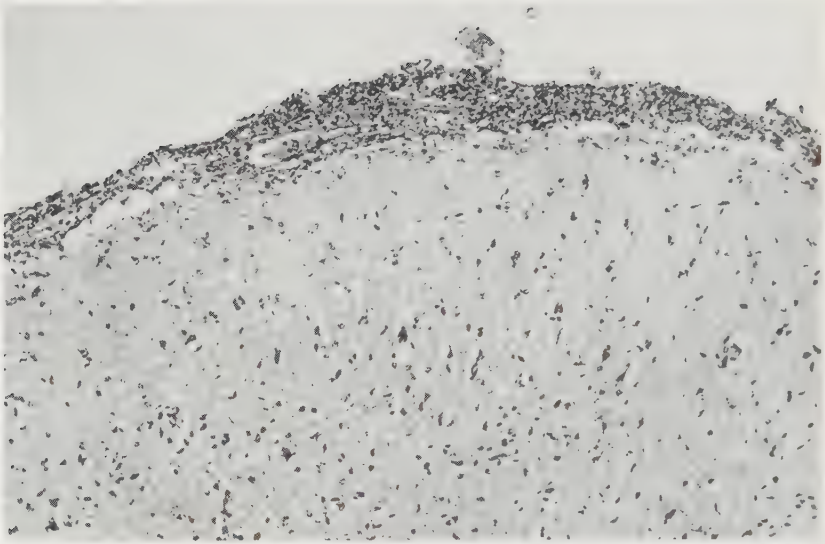

Fig. 3. Rabbit No. XII. Two days and 12 hours after infection. Eighteen hours after intravenous injection of Sp. Purulent leptomeningitis. Disintegration of exudate. Slight oedema (?) of cortical parenchyma.

Fig. 4. Rabbit No. XX. Three days and 12 hours after infection. Eighteen hours after Sp. injection. Leptomeningitis. Purulent exudate mingled with lymphocytes. Marked endovascular endothelial proliferation.

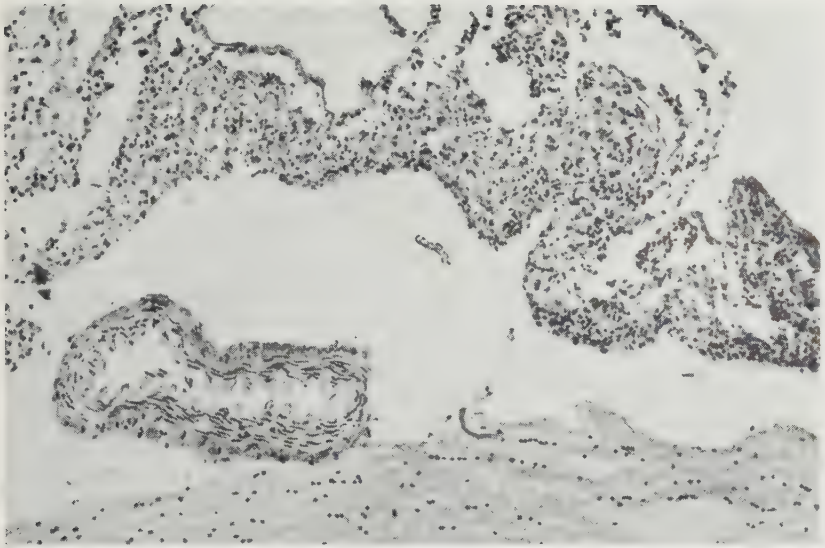

Fig. 5. Rabbit No. XXI. Four days and 12 hours after infection. Three days and 12 hours after Sp. injection. Leptomeningitis in marked decrease. Meninges thickened and infiltrated chiefly by lymphocytes and macrophages.

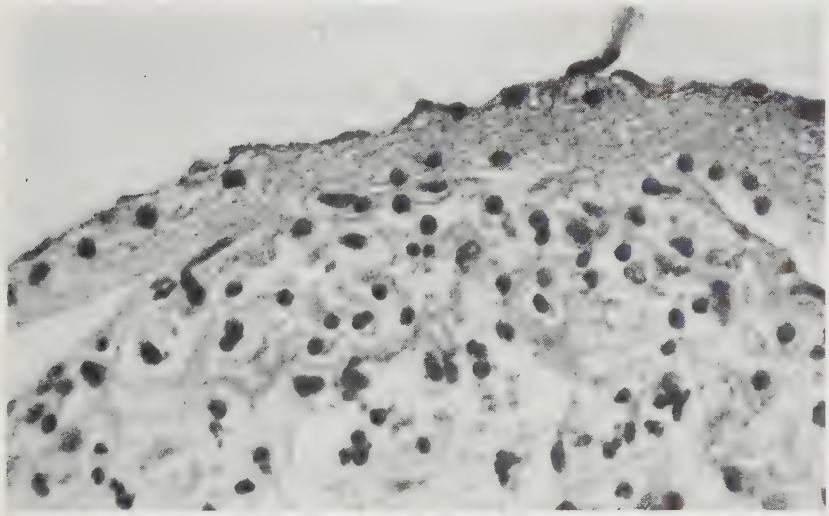

Fig. 6. Leptomeningitis of rabbit No. XXI in higher magnification. Sparse infiltration of lymphocytes in the pial sheat. Only a few polymorphonuclear cells are visible. Pial oedema.

kind has not been observed in the untreated series, and was demonstrated in one case only. It is therefore difficult to make out its connection (if any) with the Sp. therapy (fig. 4).

4 1/2-day (108 hours) meningitis.—(Sp. injected 90 hours previously.) In the sections examined the meninges are somewhat thick and fibrous, containing more or less abundant macrophages and above all small round cells. The polymorphonuclear cells are strikingly scarce. Meningeal hyperaemia. No marked changes in the brain substance (fig. 5 and 6).

Vaccinated meningitic animals.—The histological examination of the brains from the vaccinated meningitic animals showed a purulent meningitis though in general of a lower intensity. Copious phagocytosis of bacteria could be observed.

Septic animals.—The histological examination of the brains from the septic animals showed no meningitis.

Investigation of the Localization of Sp. in the Cerebral Tissue.

The animals are, after narcosis, killed in the usual way by clipping off the tip of the heart, so that they rapidly bleed to death. A troicart is then introduced into the thoracic aorta according to Broman (13) and physiological salt solution flushed through under a pressure of 130 cm of water, corresponding to the blood pressure of the rabbit. As the valves of the aorta close up, the upper half of the body can be flushed through in this way with physiological salt solution, which after a while returns clear from the heart. Flushing is then performed in the same way with 10 per cent formaline; all this is done to eliminate secondary supra- and postvital diffusion from the blood vessels as far as possible and to ensure rapid fixing. The brain is thereupon instantly removed.

To investigate the localization of Sp. in the brain author has worked out the following method.[1]

A transversal slice of the brain is laid in a small quantity of 1 N. hydrochloric acid solution for 5 minutes, and then transferred to a small quantity of 1 per cent sodium nitrite for 5 minutes. The solution is poured off, and an equal quantity of 0.2 per cent sulfamic acid solution is added. After a further 5 minutes this is poured off and 0.3 per cent ethyl-α-naphthylamine hydrochloride solution is poured on.

[1] At first the author tried to investigate the localization of Sp. in the brain by means of fluorescence-microscopic studies according to a method decribed by Richtnér (123). These experiments gave negative results, however, probably depending on too low concentration of Sp. in the brain.

In the presence of sulfanilamide derivatives certain parts of the slice at once assumes the same red colour as when the reaction was made in fluids.

For microscopic examination frozen sections from the stained brain slice are immediately cut with the microtome knife chilled in carbon dioxide. Since the chemical colour reaction only includes a rather thin superficial zone of the brainslice surface, it is necessary to chose sections within this zone. The sections are transferred to a chilled slide and mounted in diluted glycerine, after which they are ready for the microscopic examination, which is made immediately after preparation.

Macroscopic picture.—The macroscopic picture shows an intense red staining of the gray substance, whereas the white substance presents a scarcely visible red tinge (fig. 7).

Microscopic picture.—The sections showed, in lower magnification, a faint diffuse red staining of the gray substance, most pronounced in strata 2 and 4—5 of the cortex. Here the protoplasm of the nerve cells are more strongly coloured than the intercellular gray matter of the surroundings; the nuclei, on the other hand, are completely uncoloured. The gliacells and the elements of the mesenchymal tissue do not take any stain. There are also signs of diffuse red staining of the gliofibrillar substance in a more or less broad zone round a number of larger vessels in the cerebral tissue. The microscope reveals that the white substance also has some sparse and extremely faintly stained structures of an uncertain nature, possibly myelin sheats or capillaries. The condition of the meninges cannot be examined in these sections as they are not contained in the sections when using this cutting method technic.

The microscopic sections from the macroscopic red-coloured portions, and from the cortex in particular, thus showed the protoplasm of the nerve-cells to be faintly but quite distinctly stained, whereas their nuclei were completely unstained throughout. It might be supposed from this that this cellular impregnation has arisen intravitally, in other words, that Sp. really does show a distinct affinity for the ganglia cells. The following test, however, was made to control whether this affinity of the nerve cells occurs intravitally or represents a supravital or postmortal imbibition taking place during the period, less than half an hour, elapsing between the killing of the animal and the microtomic cutting.

An animal was killed in the usual way by bleeding to death; it was flushed through with physiological salt solution according to the method

given above until clear fluid ran out from the heart. The body was then left until 15 minutes after death, after which flushing was performed with an solution of Sp. containing 80 mgm per 100 ml for 15 minutes. Flushing with formaline was then made and the brain removed, on which the chemical reaction was subsequently made.

This test showed that the macroscopic stain reaction had, on the whole, the same extent as in the animals receiving the drug intravenously during life, though in the former case it was not so intense.

The sections from these preparations appeared to the naked eye faintly tinged with red, but showed microscopically no signs of nerve-cell staining, nor could any concentration of the coulur round the vessels be observed. This fact suggests that the ganglia-cell staining demonstrated in the intravital test really is due to intravital imbibition with Sp.

Summary.

The histological investigation of brains during different phases of the meningitis thus showed a continuous progression of purulent leptomeningitis, which in the advanced cases is combined also with a superficial encephalitic change of the brain cortex.

The histological picture of the meningitis in treated animals was the following: The cases receiving the longest treatment showed an increased meningeal round-cell infiltration and definite signs of healing. In one case there was endovascular proliferation.

The macroscopic chemical reaction showed that the Sp. was predominantly localized in the gray substance. As the animal's brain had been flushed through with physiological salt and formaline during life, the Sp. must therefore have penetrated out through the vessels and been deposited mainly in these parts of the cerebral tissue intravitally.

The microscopic investigation into the localization of the Sp. after the chemical stain reaction showed the drug to have a faint but neverthless distinct affinity for the protoplasm of the nerve-cells; in the light of the control tests made this must, in all probability, have arisen intravitally.

Fig. 7. Coloured photograph of a frontal slice from the brain on which the specific chemical stain reaction on sulfapyridine has been made. The picture shows a strong red colouration of the gray substance, while the white substance presents a hardly visible red tinge.

Chapter 5.

The Time for Taking Samples.

Healthy animals.—The concentration of Sp. in blood, plasma and cerebrospinal fluid was first examined in a smaller series of animals at regular intervals after injection of the drug. Diagrams were then drawn showing these concentrations during the hours immediately following infection. With the help of the diagrams it was then possible to determine the optimal juncture for the sample-taking. Several such type-curves are given on p. 48.

As figure 8 shows, the plasma concentration after *intravenous* injection rapidly attains a high starting value and sinks quickly during the first 30 minutes to about the half, after which it drops more slowly. The concentration in the spinal fluid rises more slowly, reaching its highest value after half-an-hour, after which it slowly sinks. The diagram shows that the relation between the fluid and the plasma concentration varies greatly in the course of the first hours after injection. The peak of the spinal fluid curve gives the juncture at which the passage of Sp. from plasma to fluid ceases, and constitutes a gauge of how much Sp. can pass over into the spinal fluid at the corresponding plasma concentration. The later part of the diagram shows the equilibrium brought about when both the plasma and the fluid values slowly sink. Half an hour after the injection, corresponding to the peak of the fluid curve, therefore seems to be the point of time best suited for a study of the extent to which Sp. passes over from blood to fluid. To determine the concentration of Sp. in the blood and cerebrospinal fluid after intravenous injection in a large series of animals, samples were therefore taken half-an-hour after the drug had been administered.

The state of concentration in plasma and cerebrospinal fluid after *subcutaneous* injection of the drug is shown by fig. 10. The values on the ordinate are drawn on a double scale in relation to fig. 8. The diagram shows that the plasma concentration rises fairly slowly during the first hour, reaches its highest value after

Diagrams showing the concentr. of Sp. in plasma and cerebrospinal fluid.

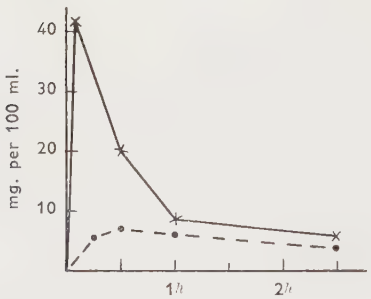

Fig. 8. Intraven. Med.—Non-inf. rabbit.

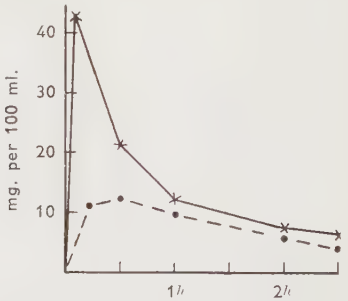

Fig. 9. Intraven. Med.—Mening. rabbit.

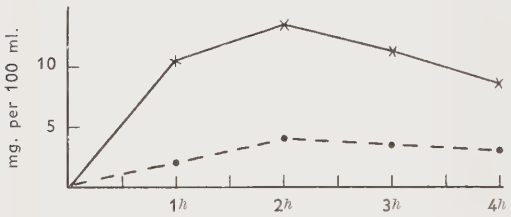

Fig. 10. Subcutan. Med.—Non-inf. rabbit.

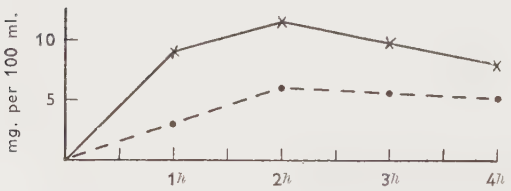

Fig. 11. Subcutan. Med.—Mening. rabbit.

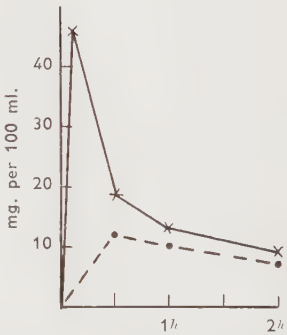

Fig. 14. Intraven. Med.—Septic rabbit.

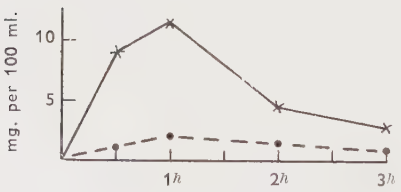

Fig. 12. Intramusc. Med.—Non-inf. rabbit.

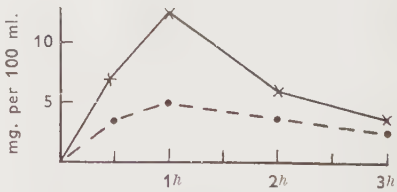

Fig. 13. Intramusc. Med.—Mening. rabbit.

——x—— the concentr. of Sp. in plasma
– – • – – » » » Sp. » cerebrospinal fluid

2 hours, and then gently sinks. The fluid concentration curve reaches considerably lower values, but otherwise it takes a similar course. Here, too, the highest value is reached after 2 hours, The figures from different animals varied more in this series than in the previous one, probably due to different rates of absorption from the subcutaneous deposits of the drug. Nevertheless, in most cases maximal concentration hade been reached after 2 hours. When the drug was injected subcutaneously, samples were therefore taken 2 hours after administration.

The state of concentration in plasma and cerebrospinal fluid after *intramuscular* injection of the drug is shown by fig. 12, on which the curves have been drawn on the same scale as the curves in fig. 10. The curve of the plasma concentration in this fig. is a kind of intermediate between corresponding curves in fig. 8 and 10. Maximal concentration in plasma and fluid is reached 1 hour after the injection of Sp. The variation in the curves from different animals was not so great in this series as after subcutaneous administration of the drug. Samples were taken 1 hour after intramuscular injection.

Naturally, it has not been possible, under the described conditions, to get an idea as to the state of the tissue concentration at different times after administering the drug to one and the same animal; instead, therefore, the concentration at different times after injection of Sp. has been studied on different animals. The values thus obtained are reproduced in the diagrams shown on p. 51. To eliminate the variation in the blood concentration and its effect on the tissue concentration in different animals, the actual figures for the tissue concentration have not been used when making the diagrams; instead the ratio has been taken between the tissue and the plasma concentration of total (i. e. sum of free and acetylated drug) Sp. at the same point in time. The ratios from different animals thus obtained have been recalculated in relation to a curve of the plasma concentration of total Sp. in one and the same animal at different junctures after the injection of Sp.

Fig. 15 shows that, after intravenous injection, the Sp. passes very rapidly into the tissue, except in the brain where the concentration shows a lower value. A certain agreement between the

curve of the brain concentration and the curve of the fluid concentration in fig. 8 is to be noted.

Fig. 17 and 19 also show that, after intramuscular and subcutaneous injection, the brain concentration is appreciably lower than that of the remaining tissue. These cases, too, show an agreement between the curves of the brain concentration and those of the fluid concentration in figs. 10 and 12.

The brain concentration from Sp. after injection in different ways thus displays an agreement with the concentration in the cerebrospinal fluid, and is lower than that in the other tissue. The curve of the concentration in other organs runs parallel to that of the plasma concentration, but on a somewhat lower level. In view of these circumstances samples of tissue have been taken at the same juncture as has been described earlier on for blood and spinal fluid.

Infected Animals.—The distribution of Sp. in the blood, cerebrospinal fluid and tissue of infected animals was investigated after making similar preliminary tests for plasma, fluid and tissue concentrations in the hours immediately following injection of the drug. The diagrams from these tests are given in figs. 9, 11, 13, 14, 16, 18, 20.

As can be seen from these diagrams, the values for the plasma concentration are more or less the same in the infected and the healthy animals. The values for the fluid concentration, on the other hand, rise throughout to higher levels in the infected animals. The values for the concentration in the brain are likewise higher in the infected animals, although the difference in specimens injected subcutaneously is negligible. The point in time for maximal fluid concentration and brain concentration in the infected animals mainly coincides with that of the healthy ones. The same is true of the other tissue, except after intravenous injection; here, after 5 minutes, the tissue concentration in the infected animals is not so high as in the healthy ones, figs. 15 and 16. From the half-hour value inclusive, and later, there is nevertheless an agreement between the concentration in the organs of the infected and the healthy animals after intravenous injection also. The taking of samples from the infected animals,

Diagrams showing the concentr. of total Sp. in Plasma and Tissue.

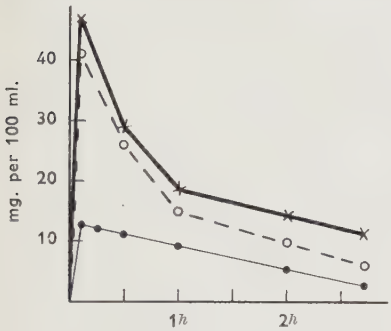

Fig. 15. Intraven. Med.—Non-inf. rabbit.

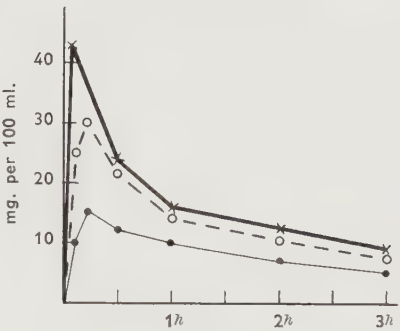

Fig. 16. Intraven. Med.—Mening. rabbit.

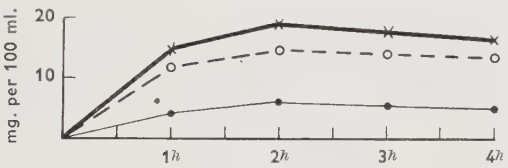

Fig. 17. Subcutan. Med.—Non-inf. rabbit.

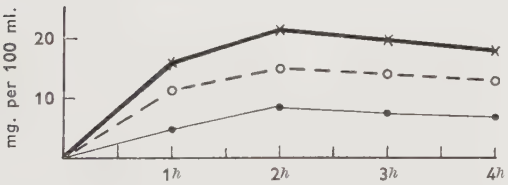

Fig. 18. Subcutan. Med.—Mening. rabbit.

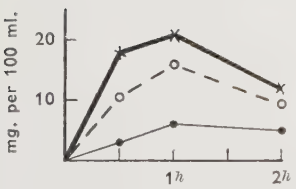

Fig. 19. Intramusc. Med.—Non-inf. rabbit.

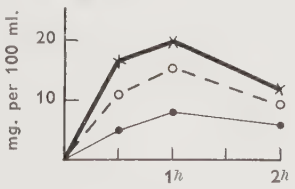

Fig. 20. Intramusc. Med.—Mening. rabbit.

—x— the concentr. of Sp. in plasma.
—•— » » » Sp. » brain.
– – o – – » mean concentr. of Sp. in the other tissue.

after injection of Sp. in different ways, has therefore been made at the same juncture as for the healthy animals as regards both blood, cerebrospinal fluid and tissue.

Chapter 6.

Distribution of Sp. in Blood, Cerebrospinal Fluid and Tissue in Healthy Animals.

Distribution between Corpuscles and Plasma.

Tables 1—3 show the concentration of free and total Sp. in whole blood and plasma, the volume percentage of plasma and corpuscles[1], and the percentage of the drug in the plasma and corpuscles. The last column gives the ratio between the concentration in blood corpuscles and in plasma. The ratios have been calculated from the percent values of the drug in corpuscles and plasma.

The distribution between corpuscles and plasma was investigated in 48 animals, which were given the drug *intravenously*. The 15 first animals in the series received 0.2 gm Sp. per kg body-weight; the remaining animals received 0.1 gm Sp. per kg body-weight. Samples were taken half-an-hour after tre drug had been injected. The values are shown in table 1. The plasma contained on an average 82 % ± 1.0 % free Sp. and 84 % ± 1.0 % total Sp.; the corpuscles contained 18 % ± 1.0 % and 16 % ± 1.0 % respectively. The mean ratio of the concentration in corpuscles and plasma was for free Sp. 0.44 ± 0.024 and for total Sp. 0.38 ± 0.025.

The distribution of Sp. in corpuscles and plasma was investigated in 19 animals after *intramuscular* injection. All the animals in this series received 0.3 gm Sp. per kg body-weight. Samples of blood were taken 1 hour after injection of Sp. The values obtained are shown in table 2. The plasma contained on

[1] The haematocrite values were determined in the usual manner; e. g. blood was drawn up in a graduated pipette. Centrifuging for 20 minutes, 3500 revolutions per minute. Double determinations were made on each blood sample.

Table 1. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Intravenous medication. Non-infected rabbits.

Rabbit No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
1	15.3	18.3	18.3	22.7	68	81	84	32	19	16	0.50	0.40
2	10.3	18.6	13.2	22.7	55	70	67	45	30	33	0.52	0.60
3	14.0	23.0	16.2	26.5	63	73	73	37	27	27	0.63	0.63
4	21.0	24.6	25.8	29.0	59	72	70	41	28	30	0.56	0.62
5	16.0	20.7	18.5	24.0	63	73	73	37	27	27	0.63	0.63
6	17.6	20.2	23.0	27.1	67	88	90	33	12	10	0.28	0.23
7	13.9	23.8	17.0	29.0	57	70	69	43	30	31	0.57	0.60
8	20.0	26.0	23.8	32.1	64	76	79	36	24	21	0.56	0.47
9	18.3	23.8	21.0	30.0	65	75	82	35	25	18	0.62	0.41
10	18.3	33.8	20.7	38.3	65	74	74	35	26	26	0.65	0.65
11	9.3	20.0	11.7	25.9	62	78	80	38	22	20	0.46	0.41
12	9.3	16.9	12.3	23.3	65	86	90	35	14	10	0.30	0.21
13	12.7	17.2	16.5	21.2	68	88	84	32	12	16	0.29	0.40
14	18.5	19.4	23.5	23.8	67	85	82	33	15	18	0.36	0.45
15	7.8	14.0	10.0	18.4	62	79	81	38	21	19	0.43	0.38
16	4.2	11.0	6.3	12.6	63	95	72	37	5	28	0.09	0.66
17	10.6	15.0	12.1	18.0	70	80	84	30	20	16	0.58	0.44
18	14.6	19.1	15.7	21.5	72	77	81	28	23	19	0.77	0.60
19	6.0	15.6	8.3	19.5	65	90	81	35	10	19	0.21	0.44
20	9.9	11.0	12.4	13.4	66	83	80	34	17	20	0.40	0.49
21	6.9	9.2	8.3	13.0	66	79	93	34	21	7	0.52	0.15
22	7.3	9.8	9.3	13.4	68	87	93	32	13	7	0.32	0.16
23	6.8	10.0	9.2	14.6	66	89	96	34	11	4	0.24	0.08
24	8.4	11.3	10.5	14.8	69	86	90	31	14	10	0.36	0.25
25	9.1	11.8	12.9	17.1	68	96	99	32	4	1	0.09	0.02
26	13.8	18.2	16.0	21.7	71	82	85	29	18	15	0.54	0.43
27	5.7	11.0	8.3	17.2	55	80	86	45	20	14	0.31	0.20
28	6.4	10.8	8.6	16.1	59	79	88	41	21	12	0.38	0.20
29	7.0	10.3	10.3	16.5	53	78	85	47	22	15	0.32	0.20
30	10.0	18.2	11.6	21.7	75	87	89	25	13	11	0.45	0.37
31	3.9	12.8	4.5	16.0	69	80	86	31	20	14	0.56	0.36
32	5.9	10.8	6.5	13.0	74	82	89	26	18	11	0.63	0.35
33	5.0	11.3	5.8	13.7	70	81	85	30	19	15	0.55	0.41
34	5.6	10.4	6.8	13.4	72	87	93	28	13	7	0.38	0.19
35	3.0	11.6	3.3	14.3	71	78	88	29	22	12	0.69	0.33
36	3.7	13.5	4.2	17.7	72	82	94	28	18	6	0.57	0.16
37	7.9	14.1	8.7	17.0	69	76	83	31	24	17	0.70	0.46
38	2.4	10.3	3.1	13.5	70	90	92	30	10	8	0.26	0.20
39	7.4	12.2	9.0	14.9	70	85	85	30	15	15	0.41	0.41
40	10.2	11.5	13.2	14.1	68	88	83	32	12	17	0.29	0.44
41	5.1	12.3	5.8	15.5	71	81	89	29	19	11	0.58	0.30
42	2.3	12.7	2.8	15.7	70	85	86	30	15	14	0.41	0.38
43	5.5	13.8	7.4	19.2	65	87	90	35	13	10	0.28	0.21
44	8.4	12.4	9.3	15.1	71	79	86	29	21	14	0.65	0.40
45	4.1	12.7	5.3	13.4	75	97	79	25	3	21	0.09	0.77
46	4.9	14.9	5.9	16.7	73	88	82	27	12	18	0.37	0.59
47	5.3	12.8	6.2	15.3	69	81	82	31	19	18	0.52	0.49
48	4.1	9.2	5.3	12.5	66	85	90	34	15	10	0.34	0.22

Table 2. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Intramuscular medication. Non-infected rabbits.

Rabbit No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
49	9.1	13.3	10.1	15.5	69	77	80	31	23	20	0.67	0.56
50	23.6	29.4	26.0	30.2	64	71	66	36	29	34	0.73	0.92
51	6.3	18.0	7.8	22.5	73	90	91	27	10	9	0.30	0.27
52	13.2	13.8	16.0	17.2	66	80	82	34	20	18	0.49	0.43
53	4.1	14.8	5.7	18.2	68	95	84	32	5	16	0.11	0.40
54	12.0	18.3	16.3	23.8	61	83	79	39	17	21	0.32	0.42
55	5.1	12.0	7.2	14.6	57	80	69	43	20	31	0.33	0.60
56	4.5	15.5	5.5	18.8	62	76	75	38	24	25	0.52	0.54
57	6.0	13.0	7.8	18.4	64	83	91	36	17	9	0.36	0.18
58	14.0	16.6	16.7	18.8	66	79	75	34	21	25	0.52	0.65
59	7.4	14.3	9.0	17.0	70	85	83	30	15	17	0.41	0.48
60	15.0	18.0	17.3	20.9	69	80	80	31	20	20	0.56	0.56
61	6.3	10.7	8.3	13.1	63	83	77	37	17	23	0.35	0.51
62	9.2	15.5	10.8	19.7	61	72	78	39	28	22	0.61	0.44
63	5.0	9.0	7.2	12.1	62	89	83	38	11	17	0.20	0.33
64	8.7	14.2	10.7	18.8	64	79	85	36	21	15	0.47	0.31
65	5.2	9.0	7.6	13.9	62	91	96	38	9	4	0.16	0.07
66	5.0	9.3	7.3	13.9	61	89	91	39	11	9	0.19	0.15
67	2.9	7.3	4.3	11.5	55	82	87	45	18	13	0.27	0.18

an average 82 % ± 1.5 % free Sp. and 82 % ± 1.8 % total Sp.; the corpuscles contained 18 % ± 1.5 % and 18 % ± 1.8 % respectively. The mean ratio of the concentration in corpuscles and plasma was for free Sp. 0.40 ± 0.041 and for total Sp. 0.42 ± 0.047. The distribution of Sp. was also investigated in 29 animals after *subcutaneous* administration. All the animals in this series were given 0.4 gm Sp. per kg. body-weight. Samples of blood were taken 2 hours later. The values obtained are shown in table 3. The plasma contained on an average 83 % ± 1.1 % free Sp. and 85 % ± 0.7 % total Sp.; the corpuscles contained 17 % ± 1.1 % and 15 % ± 0.7 % respectively. The mean ratio of the concentration in corpuscles and plasma was for free Sp. 0.36 ± 0.025 and for total Sp. 0.30 ± 0.028.

Summary.—After administration of Sp. in different ways the concentration values in whole blood for free and total Sp. were somewhat lower than the corresponding plasma values. The

plasma contained rather more than 80 per cent of both free and total Sp. in all the three series. The mean ratio of concentration in corpuscles and plasma varied somewhat with the different modes of administration. It was highest after intravenous injection and lowest after subcutaneous injection, in both cases somewhere about 0.40 for free Sp. This indicates that the concentration of free Sp. in the blood corpuscles of healthy animals is about 40 per cent of the concentration in the plasma. The corresponding values for total Sp. were, in the larger series, somewhat lower.

Concentration of Sp. in the Cerebrospinal Fluid.

The same animals as were used to investigate the distribution between corpuscles and plasma were taken for a study of the Sp. concentration in the cerebrospinal fluid from healthy animals. Samples of spinal fluid were taken immediately before the blood samples. Technical difficulties made it impossible to get such samples from more than 32 animals after intravenous injection, 16 animals after intramuscular injection and 22 animals after subcutaneous injection. The values obtained are shown in tables 4, 5 and 6. To make it easier to compare the values, which were obtained at varying blood concentrations, the elicited fluid values have in the last column been expressed as a ratio between the concentration in the cerebrospinal fluid and the plasma. After *intravenous* injection the mean of these ratio values for free Sp. was 0.37 ± 0.016 and for total Sp. 0.31 ± 0.012; after *intramuscular* injection it was 0.23 ± 0.017 and 0.22 ± 0.019 respectively, and after *subcutaneous* injection 0.35 ± 0.019 and 0.24 ± 0.014 respectively.

Summary.—The figures obtained show that animals receiving Sp. by the *intravenous and subcutaneous* route show a concentration of free Sp. in the spinal fluid of about one-third of that in the plasma at the moment of testing mentioned above. The concentration values of total Sp. are somewhat lower. Figs. 8, 10 and 12 on p. 48 show that if samples were taken earlier it would mean a lower and, if later, a higher value of the ratio between

Table 3. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Subcutaneous medication. Non-infected rabbits.

Rabbit No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
68	2.8	8.3	3.8	10.8	58	79	75	42	21	25	0.37	0.46
69	11.6	16.0	14.4	20.0	59	73	74	41	27	26	0.53	0.51
70	9.7	20.5	12.5	25.9	61	79	77	39	21	23	0.42	0.47
71	8.0	17.2	10.5	22.8	61	80	81	39	20	19	0.39	0.37
72	6.2	14.4	7.6	18 6	65	80	84	35	20	16	0.46	0.35
73	2.7	10.0	3.7	13.4	66	90	88	34	10	12	0.22	0.26
74	5.2	13.8	7.1	19.6	60	82	85	40	18	15	0.33	0.26
75	7.0	19.5	9.4	25.9	67	90	89	33	10	11	0.23	0.25
76	5.7	13.0	6.4	17.5	68	76	92	32	24	8	0.67	0.18
77	11.8	13.8	14.8	18.1	60	75	79	40	25	21	0.50	0.40
78	6.5	16.6	8.1	22.8	61	76	84	39	24	16	0.49	0.30
79	2.4	5.2	3.5	8.0	60	88	92	40	12	8	0.20	0.13
80	10.5	16.0	12.3	19.2	60	70	72	40	30	28	0.64	0.58
81	13.2	22.6	16.5	25.9	60	75	69	40	25	31	0.50	0.67
82	6.0	11.1	8.6	16.5	61	87	91	39	13	9	0.23	0.15
83	10.4	15.8	14.1	20.7	68	92	89	32	8	11	0.18	0.26
84	8.5	16.6	11.3	22.2	65	86	87	35	14	13	0.30	0.28
85	8.7	14.7	11.6	19.6	66	88	88	34	12	12	0.26	0.26
86	5.1	18.0	6.4	22.5	72	90	90	28	10	10	0.29	0.29
87	6.6	19.9	8.1	25.9	68	83	89	32	17	11	0.44	0.26
88	10.4	18.0	13.7	23.0	70	92	89	30	8	11	0.20	0.29
89	8.4	20.7	10.2	25.8	70	85	87	30	15	13	0.41	0.35
90	11.1	18.8	14.2	23.3	70	90	87	30	10	13	0.26	0.35
91	3.8	10.6	5.0	15.0	62	82	88	38	18	12	0.36	0.22
92	2.0	7.3	2.5	11.8	64	80	100	36	20	0	0.44	0.00
93	2.7	6.3	3.7	8.3	61	84	80	39	16	20	0.30	0.39
94	4.4	10.4	6.2	13.4	63	89	81	37	11	19	0.21	0.40
95	4.0	10.4	5.1	15.3	63	80	93	37	20	7	0.43	0.13
96	3.1	6.6	4.1	10.2	64	85	99	36	15	1	0.31	0.02

the concentration in fluid and plasma. Taking into account our previous discussion on the time for sample-taking, it nevertheless seems as though the procedure followed is the best one to show the amount of Sp. passing over from blood to fluid at a certain concentration in the blood.

The ratio in animals receiving the drug *intramuscularly* is lower, and the concentration in the cerebrospinal fluid about a quarter of that of the plasma at the time in question. This goes to show that the samples in these cases are taken somewhat too early. Probably, therefore, samples taken some minutes later

Table 4. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*
Intravenous medication. Non-infected rabbits.

Rabbi No.	In Plasma		In Cerebrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total
1	18.3	22.7	7.8	8.2	0.43	0.36
2	13.2	22.7	5.4	7.2	0.41	0.32
3	16.2	26.5	5.4	6.4	0.33	0.24
4	25.8	29.0	9.1	10.0	0.35	0.34
5	18.5	24.0	7.5	10.3	0.41	0.43
6	23.0	27.1	6.6	8.4	0.29	0.31
7	17.0	29.0	6.7	8.2	0.39	0.28
8	23.8	32.1	10.5	11.3	0.44	0.35
9	21.0	30.0	5.2	6.2	0.25	0.21
10	20.7	38.3	8.2	8.6	0.40	0.22
11	11.7	25.9	5.5	7.4	0.47	0.28
12	12.3	23.3	7.0	8.3	0.57	0.36
13	16.5	21.2	6.9	8.8	0.42	0.42
14	23.5	23.8	6.7	7.5	0.29	0.31
15	10.0	18.4	3.8	4.7	0.38	0.26
17	12.1	18.0	5.4	5.7	0.45	0.32
18	15.7	21.5	6.2	7.2	0.39	0.33
21	8.3	13.0	2.5	3.3	0.30	0.25
22	9.3	13.4	2.9	3.8	0.31	0.28
23	9.2	14.6	3.1	4.5	0.34	0.31
24	10.5	14.8	4.4	5.7	0.42	0.39
25	12.9	17.1	4.4	6.7	0.34	0.39
26	16.0	21.7	4.0	5.7	0.25	0.26
28	8.6	16.1	2.0	3.9	0.23	0.24
29	10.3	16.5	2.3	3.8	0.22	0.23
30	11.6	21.7	4.4	10.5	0.38	0.48
40	13.2	14.1	4.0	4.7	0.30	0.33
43	7.4	19.2	4.1	5.4	0.55	0.28
44	9.3	15.1	3.2	3.9	0.34	0.26
45	5.3	13.4	2.6	4.2	0.50	0.31

would have corresponded better to the conditions which were aimed at, and which have been obtained for animals after intravenous and subcutaneous injection. However, the same point in time has been chosen for taking samples from the infected animals, and the curves in figs. 12 & 13 do not show any essential differences between healthy and infected animals as regards the time at which the fluid curve attains its peak; a shift in the taking of samples will not, therefore, have any effect on possible differences.

Table 5. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*

Intramuscular medication. Non-infected rabbits.

Rabbit No.	In Plasma		In Cerebrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total[1]
49	10.1	15.5	2.9	5.6	0.29	0.36
50	26.0	30.2	9.0	10.2	0.35	0.34
52	16.0	17.2	4.0	5.4	0.25	0.31
53	5.7	18.2	1.1	1.9	0.19	0.10
55	7.2	14.6	1.0	2.5	0.14	0.17
56	5.5	18.8	1.6	3.1	0.29	0.16
57	7.8	18.4	2.4	3.6	0.31	0.20
58	16.7	18.8	3.4	4.8	0.20	0.26
59	9.0	17.0	2.2	3.0	0.24	0.18
60	17.3	20.9	2.0	2.9	0.12	0.14
61	8.3	13.1	1.1	3.0	0.13	0.23
62	10.8	19.7	2.9	3.0	0.27	0.15
63	7.2	12.1	1.6	2.5	0.22	0.21
64	10.7	18.8	2.6	2.8	0.24	0.15
65	7.6	13.9	2.1	6.5	0.28	0.47
67	4.3	11.5	0.8	1.8	0.18	0.16

Concentration of Sp. in Tissue.

The same animals were used for determining the concentration of Sp. in tissue after administration by different ways as were investigated previously for the distribution in blood and cerebrospinal fluid. The values obtained are seen in tables 7, 8 and 9, which also show the number of animals used in the different series. Together with the figures actually found, figures expressing the relation between the concentration in the respective organs and in the plasma have been given in the tables, in order to facilitate a comparison of the values obtained at different blood concentrations. These ratios thus give how much Sp. is to be found in the tissue, including the blood in the capillaries, in relation to the concentration in the plasma at the time in question. All the values obtained express the total Sp. content in the organs. The value of total Sp. in the plasma has therefore been used to calculate the ratio between the tissue and the plasma concentration. The mean ratios of the concentration in tissue and in plasma after *intravenous* injection were, for liver and lung

Table 6. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*

Subcutaneous medication. Non-infected rabbits.

Rabbit nr.	In Plasma		In Cerebrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total
69	14.4	20.0	3.8	5.0	0.26	0.25
70	12.5	25.9	5.8	7.6	0.46	0.29
71	10.5	22.8	2.9	4.6	0.28	0.20
72	7.6	18.6	2.9	4.2	0.38	0.23
73	3.7	13.4	1.2	2.4	0.32	0.18
76	6.4	17.5	2.3	2.9	0.36	0.17
78	8.1	22.8	4.6	6.4	0.57	0.28
79	3.5	8.0	2.0	3.3	0.57	0.41
80	12.3	19.2	4.0	6.8	0.33	0.36
81	16.5	25.9	5.2	7.0	0.32	0.27
82	8.6	16.5	2.7		0.31	
83	14.1	20.7	5.0		0.35	
84	11.3	22.2	3.1	5.7	0.27	0.26
85	11.6	19.6	3.5	5.2	0.30	0.27
88	13.7	23.0	4.7	6.2	0.34	0.27
89	10.2	25.8	3.2	5.5	0.31	0.21
91	5.0	15.0	1.4	2.7	0.27	0.18
92	2.5	11.8	1.1	2.1	0.44	0.18
93	3.7	8.3	1.1	2.1	0.30	0.25
94	6.2	13.4	1.8	2.7	0.28	0.20
95	5.1	15.3	1.8	2.6	0.34	0.17
96	4.1	10.2	1.1	1.9	0.27	0.19

0.87 ± 0.030 and 0.89 ± 0.026 respectively, for musculature and spleen 0.63 ± 0.027 and 0.65 ± 0.029 respectively, and for brain 0.39 ± 0.015. The corresponding values after *intramuscular* injection were, for liver and lung 0.96 ± 0.044 and 1.04 ± 0.038 respectively, for musculature and spleen 0.60 ± 0.032 and 0.68 ± 0.045 respectively, and for brain 0.32 ± 0.028. After *subcutaneous* injection the corresponding values were, for liver and lung 0.87 ± 0.025 and 0.94 ± 0.034 respectively, for spleen 0.66 ± 0.023, and for brain 0.30 ± 0.021. *Thus, in all three series, the concentration in the brain is considerably lower than that in the other organs; it constitutes about one-third of the plasma concentration. Liver and lung show a high concentration, approaching that of the plasma, while that of musculature and spleen is about two-thirds that of the plasma.*

Table 7. *Distribution of Sulfapyridine between Tissue and Plasma. Intravenous medication. Non-infected rabbits.*

Rabbit No.	In Plasma Total mg per 100 ml	In Brain Total mg per 100 gm	Ratio Brain value / Plasma value	In Muscle Total mg per 100 gm	Ratio Muscle value / Plasma value	In Liver Total mg per 100 gm	Ratio Liver value / Plasma value	In Spleen Total mg per 100 gm	Ratio Spleen value / Plasma value	In Lung Total mg per 100 gm	Ratio Lung value / Plasma value
1	22.7	12.3	0.54	15.7	0.69	21.2	0.93	16.8	0.74	24.6	1.08
3	26.5	11.2	0.42	18.1	0.68	26.7	1.01	20.7	0.78	27.9	1.05
4	29.0	16.2	0.56	23.4	0.81	28.3	0.98	25.1	0.87	30.6	1.06
5	24.0	11.3	0.47	17.3	0.72	22.0	0.92	19.6	0.82	24.2	1.01
6	27.1	11.6	0.43	17.6	0.65	23.3	0.86	17.6	0.65	25.1	0.93
7	29.0	10.8	0.37	19.4	0.67	25.1	0.87	20.0	0.69	26.2	0.90
8	32.1	13.8	0.43	19.7	0.61	29.5	0.92	22.9	0.71	25.1	0.78
9	30.0	12.3	0.41	16.8	0.56	24.0	0.80	16.8	0.56	28.3	0.94
10	38.3	10.2	0.27	20.1	0.52	22.9	0.60	16.8	0.44	25.1	0.66
11	25.9	9.3	0.36	19.0	0.73	25.3	0.98	20.6	0.80	25.3	0.98
12	23.3	8.0	0.34	12.2	0.52	12.4	0.53			15.7	0.67
13	21.2	9.7	0.46			19.0	0.90	12.4	0.58	18.5	0.87
14	23.8	12.0	0.50	16.8	0.71	21.2	0.89	17.4	0.73	22.7	0.95
15	18.4	7.3	0.40	16.5	0.90	18.4	1.00	16.0	0.87	21.1	1.15
16	12.6	3.0	0.24			7.4	0.59	5.3	0.42	8.3	0.66
18	21.5	9.6	0.45	15.0	0.70	26.4	1.23	14.3	0.67	22.9	1.07
19	19.5	5.9	0.30	10.3	0.53	16.3	0.84	9.0	0.46	14.7	0.75
20	13.4	5.0	0.37	7.5	0.56	13.3	0.99	9.2	0.69	11.3	0.84
21	13.0	4.3	0.33	7.6	0.59	10.3	0.80	7.6	0.59	9.8	0.76
22	13.4	5.0	0.37	7.3	0.54	13.0	0.97	8.1	0.60	10.4	0.78
23	14.6	5.5	0.38	6.5	0.45	11.7	0.80	7.0	0.48	12.5	0.86
24	14.8	5.7	0.38	8.5	0.58	12.5	0.85	8.4	0.57	12.5	0.85
25	17.1	5.5	0.32	10.3	0.60	13.8	0.81	9.8	0.57	13.7	0.80
26	21.7	10.0	0.46	21.3	0.98	24.6	1.13	21.2	0.98	24.0	1.11
27	15.5	3.8	0.25	6.0	0.39	9.0	0.58	6.3	0.41	12.0	0.77
28	16.1	4.9	0.30	7.3	0.45	11.2	0.70	8.5	0.53	15.2	0.94
29	16.5	4.2	0.25	8.0	0.48	11.5	0.70	8.1	0.49	11.1	0.67
30	21.7	8.8	0.41	16.7	0.77	21.2	0.98	17.4	0.80	20.1	0.93
40	14.1	5.9	0.42	10.3	0.73	13.8	0.98	11.4	0.81	13.0	0.92

Chapter 7.

Distribution of Sp. in Blood, Cerebrospinal Fluid and Tissue in Infected Animals.

Distribution between Corpuscles and Plasma.

For the investigation of the Sp. distribution between corpuscles and plasma in meningitic animals, the whole blood and plasma values were determined in 33 animals after *intravenous* injection. The first 18 animals were given 0.2 gm Sp. per kg

Table 8. *Distribution of Sulfapyridine between Tissue and Plasma. Intramuscular medication. Non-infected rabbits.*

Rabbit No.	In Plasma Total mg per 100 ml	In Brain Total mg per 100 gm	Ratio Brain value / Plasma value	In Muscle Total mg per 100 gm	Ratio Muscle value / Plasma value	In Liver Total mg per 100 gm	Ratio Liver value / Plasma value	In Spleen Total mg per 100 gm	Ratio Spleen value / Plasma value	In Lung Total mg per 100 gm	Ratio Lung value / Plasma value
49	15.5	4.2	0.27	9.3	0.60	14.0	0.90	14.0	0.90	17.9	1.15
50	30.2	19.4	0.64	19.0	0.63	37.0	1.23			42.6	1.41
51	22.5	4.6	0.20	11.2	0.50	18.5	0.82	13.8	0.61	19.7	0.88
52	17.2	8.7	0.50	11.5	0.67	17.3	1.01	10.9	0.63	17.3	1.01
53	18.2	3.9	0.21	12.3	0.68	15.7	0.86	9.8	0.54	17.3	0.95
54	23.8	7.8	0.33	11.3	0.47	20.0	0.84	15.9	0.67	25.8	1.08
55	14.6	2.8	0.19	5.5	0.38	9.7	0.66	6.9	0.47	12.5	0.86
56	18.8	5.2	0.28	10.5	0.56	18.5	0.98	11.5	0.61	17.6	0.94
57	18.4	5.5	0.30	9.6	0.52	16.0	0.87	12.4	0.67	17.1	0.93
58	18.8	7.2	0.38	11.2	0.60	20.0	1.06	17.6	0.94	19.9	1.06
59	17.0	5.2	0.31	15.0	0.88	15.6	0.92	14.7	0.86	16.3	0.96
60	20.9	10.8	0.52	16.3	0.78	23.3	1.11	21.2	1.01	24.4	1.17
61	13.1	5.2	0.40	8.3	0.64	12.9	0.99	9.4	0.72	15.7	1.20
62	11.1	2.8	0.25	6.1	0.55	8.1	0.73	6.7	0.60	8.2	0.74
63	12.1	4.7	0.38	11.0	0.91	17.2	1.42	12.4	1.02	18.1	1.50
64	18.8	4.6	0.24	9.3	0.49	16.3	0.87	11.0	0.59	18.5	0.98
65	13.9	3.7	0.27	6.7	0.48	12.8	0.92	6.6	0.47	13.7	0.99
66	13.9	2.9	0.21	7.1	0.51	17.4	1.25	14.2	1.02	13.9	1.00
67	13.0	2.6	0.20	6.7	0.52	9.9	0.76	8.8	0.68	11.7	0.90

Table 9. *Distribution of Sulfapyridine between Tissue and Plasma. Subcutaneous medication. Non-infected rabbits.*

Rabbit No.	In Plasma Total mg per 100 ml	In Brain Total mg per 100 gm	Ratio Brain value / Plasma value	In Muscle Total mg per 100 gm	Ratio Muscle value / Plasma value	In Liver Total mg per 100 gm	Ratio Liver value / Plasma value	In Spleen Total mg per 100 gm	Ratio Spleen value / Plasma value	In Lung Total mg per 100 gm	Ratio Lung value / Plasma value
68	10.8	1.4	0.13	2.8	0.26	7.3	0.68	5.9	0.55	6.5	0.60
69	18.0	6.0	0.33			14.0	0.78	10.2	0.57	15.2	0.84
70	25.9	8.4	0.32	19.6	0.76	24.2	0.93	21.8	0.84	27.3	1.05
71	22.8	7.8	0.34	13.7	0.60	20.6	0.91	15.2	0.67	19.6	0.86
72	18.6	5.0	0.27	10.0	0.54	17.4	0.94	10.8	0.58	16.8	0.90
73	13.4	2.4	0.18	6.5	0.49	9.2	0.69	8.4	0.63	10.2	0.76
74	19.6	4.5	0.23	11.5	0.59	17.9	0.91	12.3	0.63	21.8	1.11
75	25.9	7.5	0.29	17.4	0.67	22.9	0.88	18.5	0.71	27.8	1.07
76	17.5	4.2	0.24			13.8	0.79	10.0	0.57	15.2	0.87
77	18.1	7.4	0.41			14.8	0.82	11.4	0.63	19.6	1.08
78	22.8	6.6	0.29	15.7	0.69	24.0	1.05	18.5	0.81	21.8	0.96
79	8.0	2.3	0.28	4.0	0.50	6.8	0.85	5.5	0.69	8.3	1.04
80	19.2	7.8	0.40			18.8	0.98	14.8	0.77	19.6	1.02
81	25.9	9.8	0.38			21.8	0.84	16.7	0.64	24.0	0.93
82	16.5	4.1	0.25			14.0	0.85	8.8	0.53	15.2	0.92
83	20.7	8.7	0.42			19.8	0.96	13.7	0.66	20.6	1.00

Table 10. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Intravenous medication. Meningitic rabbits.

Rabbit No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free	Total	Free	Total		Free	Total		Free	Total		
	mg per 100 ml	mg per 100 ml	mg per 100 ml	mg per 100 ml	%	%	%	%	%	%	Free	Total
97	21.6	23.2	23.7	25.7	59	65	65	41	35	35	0.77	0.77
98	15.5	21.4	17.0	24.0	55	60	62	45	40	38	0.82	0.75
99	26.1	29.2	29.3	36.3	60	67	75	40	33	25	0.74	0.50
100	18.6	23.2	20.5	26.0	70	77	78	30	23	22	0.70	0.66
101	14.0	20.0	16.0	23.6	67	77	79	33	23	21	0.61	0.54
102	20.5	26.0	21.7	28.0	68	72	73	32	28	27	0.83	0.79
103	25.0	28.8	25.9	33.1	65	67	75	35	33	25	0.92	0.62
104	19.4	27.2	21.2	32.1	63	69	74	37	31	26	0.77	0.60
105	18.8	21.0	21.2	26.7	59	67	75	41	33	25	0.71	0.48
106	27.2	35.9	28.0	36.7	70	72	72	30	28	28	0.91	0.91
107	23.0	30.7	25.9	34.7	64	72	72	36	28	28	0.69	0.69
108	29.5	32.7	32.1	48.6	64	70	95	36	30	5	0.76	0.09
109	21.0	22.2	23.8	30.0	64	73	86	36	27	14	0.66	0.29
110	20.0	22.7	23.8	26.7	66	79	78	34	21	22	0.52	0.55
111	9.9	18.3	11.5	21.7	64	74	76	36	26	24	0.62	0.56
112	18.3	23.9	18.6	25.9	70	71	76	30	29	24	0.95	0.74
113	17.9	21.0	18.3	23.3	66	67	73	34	33	27	0.96	0.72
114	16.6	24.9	19.2	32.7	64	74	84	36	26	16	0.62	0.34
115	8.7	15.8	11.6	21.2	60	80	81	40	20	19	0.38	0.35
116	11.5	18.0	13.3	21.5	70	81	84	30	19	16	0.55	0.44
117	19.1	21.8	23.0	28.4	63	76	82	37	24	18	0.54	0.37
118	17.8	18.3	21.2	23.8	66	79	86	34	21	14	0.52	0.32
119	16.0	18.8	17.5	22.8	70	77	85	30	23	15	0.70	0.41
120	20.5	21.6	21.7	24.6	69	73	79	31	27	21	0.82	0.59
121	16.5	19.2	17.2	22.3	74	77	86	26	23	14	0.85	0.46
122	21.0	21.4	21.0	21.9	70	70	72	30	30	28	1.00	0.91
123	15.5	19.7	17.5	22.7	67	76	77	33	24	23	0.64	0.61
124	15.0	18.6	17.0	20.2	69	78	75	31	22	25	0.63	0.74
125	21.0	21.0	22.3	23.3	70	74	78	30	26	22	0.82	0.66
126	5.8	14.4	6.7	16.5	70	81	80	30	19	20	0.55	0.58

body-weight, the others received 0.1 gm per kg body-weight. The values are shown in table 10. The plasma contained, on an average, 73 % ± 0.9 % free Sp. and 78 % ± 1.2 % total Sp.; the corpuscles contained 27 % ± 0.9 % and 22 % ± 1.2 % respectively. The mean ratios of the concentration in corpuscles and in plasma were, for free Sp. 0.72 ± 0.028 and for total Sp. 0.57 ± 0.035.

The distribution of Sp. between corpuscles and plasma in meningitic animals was investigated in 15 animals after *intra-*

Table 11. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Intramuscular medication. Meningitic rabbits.

Rabbit No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
130	5.3	9.8	6.3	13.1	69	84	92	31	16	8	0.42	0.19
131	4.7	10.2	5.1	12.9	70	76	89	30	24	11	0.74	0.29
132	5.0	9.2	5.4	11.5	69	75	86	31	25	14	0.74	0.36
133	4.9	11.5	5.1	14.6	67	70	85	33	30	15	0.87	0.36
134	22.2	23.3	23.0	24.0	65	67	67	35	33	33	0.92	0.92
135	7.9	12.2	8.9	12.8	70	79	73	30	21	27	0.62	0.86
136	19.6	20.0	21.1	22.2	70	75	78	30	25	22	0.78	0.66
137	8.2	9.7	9.4	10.9	71	81	80	29	19	20	0.58	0.61
138	8.2	12.5	10.0	15.3	65	79	80	35	21	20	0.49	0.46
139	8.0	12.3	9.3	16.3	65	76	86	35	24	14	0.56	0.30
140	11.0	11.3	13.2	14.0	67	80	83	33	20	17	0.51	0.42
141	5.7	12.6	6.4	16.0	70	79	89	30	21	11	0.62	0.29
142	12.4	14.2	15.8	17.2	62	79	75	38	21	25	0.43	0.54
143	8.2	10.0	8.9	13.3	61	66	81	39	34	19	0.81	0.37
144	6.3	9.3	7.7	14.2	59	72	90	41	28	10	0.56	0.16

muscular injection, and in 21 after subcutaneous injection of Sp. In the first case the animals received 0.3 gm Sp. per kg body-weight, and in the second 0.4 gm per kg body-weight. The values obtained are shown in tables 11—12. After intramuscular injection the plasma contained, on an average, 76 % ± 1.3 % free Sp. and 82 % ± 1.8 % total Sp.; the corpuscles contained 24 % ± 1.3 % and 18 % ± 1.8 % respectively. The mean ratios of the concentration in corpuscles and in plasma in this series were, for free Sp. 0.64 ± 0.041 and for total Sp. 0.45 ± 0.058. After *subcutaneous* injection the plasma contained, on an average, 75 % ± 1.5 % free Sp. and 78 % ± 2.0 % total Sp.; corresponding figures for the corpuscles were 25 % ± 1.5 % and 22 % ± 2.0 %. The mean ratios of the concentration in corpuscles and in plasma were, for free Sp. 0.59 ± 0.043 and for total Sp. 0.52 ± 0.056.

For the investigation of the distribution between corpuscles and plasma in s e p t i c animals, the whole blood and plasma values were determined in 24 animals after intravenous injection. All the animals in this series received 0.2 gm Sp. per kg body-weight. The values are given in table 13. The plasma

Table 12. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Subcutaneous medication. Meningitic rabbits.

Rabbit No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 mg	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
145	3.4	6.7	4.2	10.1	58	72	87	42	28	13	0.54	0.21
146	6.8	16.5	9.4	23.3	59	82	83	41	18	17	0.32	0.29
147	11.0	25.0	12.9	26.1	65	76	68	35	24	32	0.59	0.87
148	9.9	18.4	10.8	23.8	63	69	81	37	31	19	0.76	0.40
149	13.8	21.8	17.0	26.0	61	75	73	39	25	27	0.52	0.58
150	12.7	20.7	14.9	23.6	61	72	70	39	28	30	0.61	0.67
151	17.4	26.1	18.1	28.0	60	62	64	40	38	36	0.92	0.84
152	6.5	16.8	8.0	18.9	63	78	71	37	22	29	0.48	0.69
153	5.1	13.3	5.2	15.3	70	71	81	30	29	19	0.95	0.55
154	2.4	7.6	3.1	10.3	65	84	88	35	16	12	0.35	0.25
155	5.6	8.8	7.7	11.8	65	89	87	35	11	13	0.23	0.28
156	5.6	14.0	7.2	19.7	62	80	87	38	20	13	0.41	0.24
157	25.0	27.2	27.7	30.1	59	65	65	41	35	35	0.77	0.77
158	13.2	23.3	15.5	28.0	65	76	78	35	24	22	0.59	0.52
159	23.3	31.6	25.9	34.2	66	73	71	34	27	29	0.72	0.79
160	6.9	17.4	8.2	23.8	62	74	85	38	26	15	0.57	0.29
161	17.7	25.0	19.1	26.9	76	82	82	24	18	18	0.70	0.70
162	11.0	17.2	12.6	18.8	64	73	70	36	27	30	0.66	0.76
163	2.8	5.6	3.3	8.2	63	74	92	37	26	8	0.60	0.15

contained, on an average, 77 % ± 1.0 % free Sp. and 79 % ± 1.3 % total Sp.; the corpuscles had 23 % ± 1.0 % and 21 % ± 1.3 % respectively. The mean ratios of the concentration in corpuscles and in plasma were 0.74 ± 0.024 for free Sp. and 0.67 ± 0.042 for total Sp.

The distribution of Sp. between corpuscles and plasma was also investigated in 33 vaccinated animals, of which 17 had been infected subarachnoidally. In the latter the values for whole blood and plasma were determined after intravenous injection of 0.2 gm Sp. per kg body-weight; the remaining received 0.1 gm Sp. per kg body-weight. The values obtained are given in tables 14—15.

In the vaccinated meningitic animals the plasma contained, on an average, 74 % ± 1.1 % free Sp. and 77 % ± 1.3 % total Sp.; the corpuscles had 26 % ± 1.1 % and 23 % ± 1.3 % respectively. The mean ratio of the concentration in corpuscles and in plasma in this series was for free Sp. 0.81 ±

Table 13. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Intravenous medication. Septic rabbits.

Rabbit No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free	Total	Free	Total		Free	Total		Free	Total		
	mg per 100 ml	mg per 100 ml	mg per 100 ml	mg per 100 ml	%	%	%	%	%	%	Free	Total
164	5.8	15.5	6.2	19.3	72	77	90	28	23	10	0.77	0.29
165	10.4	17.7	11.9	21.7	67	77	82	33	23	18	0.61	0.45
166	16.6	18.8	17.0	19.7	75	77	79	25	23	21	0.90	0.80
167	9.2	15.8	9.6	17.0	76	79	82	24	21	18	0.84	0.70
168	11.1	21.0	12.5	24.0	66	74	75	34	26	25	0.68	0.65
169	21.5	27.1	22.9	29.9	75	80	83	25	20	17	0.75	0.62
170	13.5	22.7	14.8	25.4	74	81	83	26	19	17	0.67	0.58
171	3.1	11.0	3.6	14.0	67	78	85	33	22	15	0.57	0.36
172	11.0	17.6	12.4	21.6	66	74	81	34	26	19	0.68	0.46
173	12.4	21.0	14.2	24.3	66	76	76	34	24	24	0.61	0.61
174	21.9	29.2	23.4	30.0	70	75	72	30	25	28	0.78	0.91
175	21.6	21.9	23.9	24.6	69	76	78	31	24	22	0.70	0.63
176	12.0	18.8	14.5	18.4	67	81	66	33	19	34	0.48	1.05
177	9.5	19.5	10.2	20.7	71	76	75	29	24	25	0.77	0.82
178	15.8	20.5	18.0	23.8	55	63	64	45	37	36	0.72	0.69
179	21.4	25.5	23.5	27.5	69	76	74	31	24	26	0.70	0.78
180	14.9	20.3	15.7	21.8	73	77	78	27	23	22	0.81	0.76
181	15.1	22.9	15.7	23.9	74	77	77	26	23	23	0.85	0.85
182	9.5	18.7	10.0	22.5	66	69	79	34	31	21	0.87	0.52
183	14.4	24.0	15.5	25.0	72	78	75	28	22	25	0.73	0.86
184	16.7	19.4	18.3	22.3	76	83	87	24	17	13	0.65	0.47
185	10.6	19.4	11.3	22.1	78	83	89	22	17	11	0.73	0.44
186	7.4	13.5	7.4	16.0	71	71	84	29	29	16	1.00	0.47
187	4.7	11.3	4.8	10.7	84	86	80	16	14	20	0.86	1.31

0.038 and for total Sp. 0.68 ± 0.045. In the *vaccinated, not infected* animals the plasma contained, on an average, 80 % ± 1.1 % free Sp. and 85 % ± 1.8 % total Sp.; the corpuscles showed 20 % ± 1.1 % and 15 % ± 1.8 % respectively. The mean ratios of the concentration in corpuscles and in plasma in this series were 0.54 ± 0.029 for free Sp. and 0.39 ± 0.050 for total Sp.

Summary.—After administration of Sp. by all three routes —intravenous, intramuscular and subcutaneous— the whole blood concentration values for free and total Sp. were somewhat lower than the corresponding plasma values. The plasma contained about 70—80 per cent of both free and total Sp. in all the series. The mean ratios of the concentration of free Sp. in corpuscles and in the plasma varied somewhat according to the mode

Table 14. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Intravenous medication. Vaccinated rabbits, not infected.

Rabbit No.	Aggl. titer	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Cor-puscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
		Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
188	1 : 8	13.2	20.7	15.2	22.5	71	82	77	29	18	23	0.54	0.73
189	1 : 8	10.9	20.5	13.9	26.0	65	83	82	35	17	18	0.38	0.41
190	1 : 32	12.2	17.0	14.5	22.7	64	76	85	36	24	15	0.56	0.31
191	1 : 64	17.4	17.7	19.2	21.1	64	71	76	36	29	24	0.73	0.56
192	1 : 64	18.0	23.0	21.1	25.9	66	77	74	34	23	26	0.58	0.68
193	1 : 16	6.0	15.8	7.9	21.2	56	74	75	44	26	25	0.45	0.42
194	1 : 16	10.5	16.2	12.2	19.5	71	82	86	29	18	14	0.54	0.40
195	1 : 32	7.5	16.0	8.5	20.5	66	75	85	34	25	15	0.65	0.34
196	1 : 16	10.2	15.8	12.2	22.8	65	78	94	35	22	6	0.52	0.12
197	1 : 16	4.4	14.4	5.2	18.6	72	85	93	28	15	7	0.45	0.19
198	1 : 32	3.7	13.5	4.2	17.7	72	82	94	28	18	6	0.57	0.16
199	1 : 16	11.4	18.2	13.0	21.7	73	83	87	27	17	13	0.55	0.40
200	1 : 32	6.7	14.6	7.1	18.6	73	77	93	27	23	7	0.81	0.20
201	1 : 16	22.2	28.9	25.9	30.5	67	78	71	33	22	29	0.57	0.83
202	1 : 16	9.1	15.1	11.2	18.9	70	86	88	30	14	12	0.38	0.32
203	1 : 32	7.5	15.1	9.3	19.8	69	86	90	31	14	10	0.36	0.25
204	1 : 16	3.9	14.8	4.6	19.6	68	80	90	32	20	10	0 53	0.24

Table 15. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Intravenous medication. Vaccinated meningitic rabbits.

Rabbit No.	Aggl. titer	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Cer-puscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
		Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
205	1 : 16	15.8	21.8	16.0	25.8	67	68	79	33	32	21	0.96	0.54
206	1 : 32	22.9	25.2	24.0	28.0	70	73	78	30	27	22	0.86	0.66
207	1 : 32	27.4	28.3	27.4	29.4	70	70	73	30	30	27	1.00	0.86
208	1 : 64	26.0	26.3	26.0	29.5	74	74	83	26	26	17	1.00	0.58
209	1 : 32	18.0	26.6	19.5	27.7	72	78	75	28	22	25	0.73	0.86
210	1 : 16	19.6	23.7	20.9	25.0	71	76	75	29	24	25	0.77	0.82
211	1 : 64	23.8	26.5	24.9	26.1	66	69	65	34	31	35	0.87	1.04
212	1 : 16	19.7	34.9	20.7	39.0	68	71	76	32	29	24	0.87	0.67
213	1 : 64	13.6	23.3	13.9	26.4	68	70	77	32	30	23	0.91	0.64
214	1 : 64	25.2	27.2	28.0	30.1	66	73	73	34	27	27	0.72	0.72
215	1 : 32	22.2	23.9	24.9	28.0	74	83	87	26	17	13	0.58	0.42
216	1 : 64	18.8	22.7	21.2	25.9	68	77	78	32	23	22	0.64	0.60
217	1 : 64	23.9	24.4	25.9	27.8	64	69	73	36	31	27	0.80	0.66
218	1 : 16	24.4	26.1	25.9	28.5	68	72	74	32	28	26	0.83	0.75
219	1 : 32	25.0	27.7	25.9	28.5	71	74	73	29	26	27	0.86	0.91
220	1 : 64	20.0	21.6	20.9	28.0	67	70	87	33	30	13	0.87	0.30
221	1 : 32	13.8	19.4	16.5	22.8	70	84	82	30	16	18	0.44	0.51

Table 16. *Tabulation of the distribution of Sulfapyridine in blood in non-infected and infected rabbits respectively.*

Means of the ratios between the concentration of sulfapyridine in corpuscles and in plasma.

Intravenous medication.

Non-infected rabbits		Meningitic rabbits		Septic rabbits	
Free	Total	Free	Total	Free	Total
0.44 ± 0.024	0.38 ± 0.025	0.72 ± 0.028	0.57 ± 0.035	0.74 ± 0.024	0.67 ± 0.042
Difference as against non-infected rabbits		0.28 ± 0.037	0.19 ± 0.043	0.30 ± 0.034	0.29 ± 0.049

Non-infected rabbits		Vaccinated rabbits, not infected		Vaccinated meningitic rabbits	
Free	Total	Free	Total	Free	Total
0.44 ± 0.024	0.38 ± 0.025	0.54 ± 0.029	0.39 ± 0.050	0.81 ± 0.038	0.68 ± 0.045
Difference as against non-infected rabbits		0.10 ± 0.038	0.01 ± 0.056	0.37 ± 0.045	0.30 ± 0.051

Intramuscular medication.

Non-infected rabbits		Meningitic rabbits	
Free	Total	Free	Total
0.40 ± 0.041	0.42 ± 0.047	0.64 ± 0.041	0.45 ± 0.058
Difference as against non-infected rabbits		0.24 + 0.058	0.03 ± 0.075

Subcutaneous medication.

0.36 ± 0.025	0.30 ± 0.028	0.59 ± 0.043	0.52 ± 0.056
Difference as against non-infected rabbits		0.23 ± 0.050	0.22 ± 0.062

of administration of Sp. It was highest after intravenous, and lowest after subcutaneous injection. The ratio for total Sp. is, in general, somewhat lower than that for the free drug. After intravenous injection the concentration of free Sp. in the corpuscles of infected animals constitutes about 75 per cent of that of the plasma. After intramuscular and subcutaneous injection the corresponding values are somewhat lower.

Table 17. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*

Intravenous medication. Meningitic rabbits.

Rabbit No.	In Plasma		In Cerebrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total
100	20.5	26.0	15.9	15.9	0.78	0.61
102	21.7	28.0	12.5	14.5	0.58	0.52
103	25.9	33.1	10.7	11.4	0.41	0.34
104	21.2	32.1	12.3	14.0	0.58	0.44
105	21.2	26.7	9.1	9.8	0.43	0.37
107	25.9	34.7	14.8	19.2	0.57	0.55
108	32.1	48.6	15.7	15.9	0.49	0.33
109	23.8	30.0	13.5	14.5	0.57	0.48
110	23.8	26.7	9.6	9.6	0.40	0.36
111	11.5	21.7	9.7	10.1	0.84	0.47
112	18.6	25.9	11.1	13.0	0.59	0.50
113	18.3	23.3	10.5	11.0	0.58	0.47
114	19.2	32.7	10.3	11.7	0.54	0.36
115	11.6	21.2	8.5	11.0	0.73	0.52
116	13.3	21.5	6.4	7.2	0.48	0.33
117	23.0	28.4	9.1	16.9	0.40	0.60
118	21.2	23.8	10.3	11.0	0.49	0.46
119	17.5	22.8	9.1	10.6	0.52	0.47
120	21.7	24.6	11.1	13.9	0.51	0.57
122	21.0	21.9	11.6	13.8	0.55	0.63
123	17.5	22.7	9.2	10.2	0.53	0.45
124	17.0	20.2	10.1	12.5	0.59	0.62
125	22.3	23.3	10.4	11.5	0.47	0.49
126	6.7	16.5	3.0	3.4	0.45	0.21

To make the differences in the values of healthy and infected animals clearer, the mean ratios of the corpuscles and the plasma concentration in all series have been brought together in table 16, where the computed differences are also given. The table shows that the mean ratio of *the concentration of Sp. in corpuscles* and in plasma *in the infected animals has a higher value* than in the non-infected animals, so that a statistically significant difference is obtained for both free and total Sp. except for total Sp. after intramuscular medication. Differences have been computed for the vaccinated meningitic animals as against both healthy animals and vaccinated but not infected animals. No significant difference was obtained between the last two groups.

Table 18. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*
Intramuscular medication. Meningitic rabbits.

Rabbit No.	In Plasma		In Cerebrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total
130	6.3	13.1	2.3	4.3	0.37	0.33
134	23.0	24.0	9.6	10.5	0.42	0.44
135	8.9	12.8	3.1	4.7	0.35	0.37
136	21.1	22.2	7.4	9.5	0.35	0.43
137	9.4	10.9	3.4	5.0	0.36	0.46
138	10.0	15.3	3.1	4.7	0.31	0.31
139	9.3	16.3	3.8	5.7	0.41	0.35
140	13.2	14.0	4.7	5.8	0.36	0.41
141	6.4	16.0	2.8	7.7	0.44	0.48
142	15.8	17.2	4.0	4.2	0.25	0.24
143	8.9	13.3	2.6	3.7	0.29	0.28

Concentration of Sp. in the Cerebrospinal Fluid.

Determination of the Sp. concentration in the cerebrospinal fluid of meningitic rabbits was carried out on the same animals as were used for determining the concentration in corpuscles and plasma. 24 samples of fluid were obtained from animals after intravenous injection of Sp., 11 samples after intramuscular and 11 after subcutaneous injection.

For the investigation of the Sp. concentration in cerebrospinal fluid of septic animals, the fluid concentration was determined in the same animals as were used to determine the concentration in corpuscles and plasma. 23 samples were obtained from these animals.

Samples of cerebrospinal fluid were obtained from all the vaccinated but not infected animals, whereas only 12 were obtained from the vaccinated meningitic animals.

The values obtained are shown in tables 17—22. It is seen that the mean ratios of the concentration in fluid and in plasma were, for free Sp. 0.55 ± 0.023, 0.36 ± 0.017, 0.46 ± 0.038 for meningitic animals after intravenous, intramuscular and subcutaneous injection respectively, and for total Sp. 0.46 ± 0.022, 0.37 ± 0.024, 0.32 ± 0.044. For septic animals the mean ratio

Table 19. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*

Subcutaneous medication. Meningitic rabbits.

Rabbit No.	In Plasma		In Cerebrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total
146	9.4	23.3	2.8	3.6	0.30	0.15
147	12.9	26.1	7.8	10.6	0.60	0.41
148	10.8	23.8	4.6	8.2	0.43	0.34
150	14.9	23.6	10.6	16.4	0.71	0.69
151	18.1	28.0	8.2	8.7	0.45	0.31
153	5.2	15.3	1.9	3.3	0.37	0.22
154	3.1	10.3	1.4	2.7	0.46	0.26
160	8.2	23.8	4.0	5.2	0.49	0.22
161	19.1	26.9	10.3	11.3	0.54	0.42
162	12.6	18.8	3.8	4.9	0.30	0.26
163	3.3	8.2	1.2	2.2	0.36	0.27

Table 20. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*

Intravenous medication. Septic rabbits.

Rabbit No.	In Plasma		In Cerobrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total
164	6.2	19.3	4.1	5.7	0.67	0.30
165	11.9	21.7	6.7	8.2	0.56	0.38
166	17.0	19.7	8.1	9.3	0.47	0.47
167	9.6	17.0	6.6	7.7	0.68	0.45
168	12.5	24.0	7.4	9.5	0.59	0.40
169	22.9	29.9	10.0	11.5	0.44	0.38
170	14.8	25.4	9.0	10.3	0.61	0.41
171	3.6	14.0	3.0	5.2	0.83	0.37
172	12.4	21.6	6.7	8.6	0.54	0.40
173	14.2	24.3	7.1	8.3	0.50	0.34
174	23.4	30.0	12.2	13.6	0.52	0.45
175	23.9	24.6	10.4	11.6	0.44	0.47
176	14.5	18.4	6.7	8.2	0.46	0.44
177	10.2	20.7	5.7	7.5	0.56	0.36
178	18.0	23.8	8.3	9.0	0.46	0.38
179	23.5	27.5	8.8	9.8	0.37	0.36
180	15.7	21.8	8.0	8.8	0.51	0.40
181	15.7	23.9	8.7	10.5	0.55	0.44
182	10.0	22.5	6.5	7.2	0.65	0.32
183	15.5	25.0	8.5	9.5	0.55	0.38
184	18.3	22.3	9.4	15.2	0.51	0.68
185	11.3	22.1	6.2	7.4	0.55	0.33
186	7.4	16.0	3.3	4.1	0.45	0.26

Table 21. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*

Intravenous medication. Vaccinated rabbits, not infected.

Rabbit No.	In Plasma		In Cerebrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total
188	15.2	22.5	6.0	7.1	0.39	0.32
189	13.9	26.0	6.2	7.7	0.45	0.30
190	14.5	22.7	2.3	2.9	0.16	0.13
191	19.2	21.1	5.9	7.1	0.31	0.34
192	21.1	25.9	9.0	10.7	0.43	0.41
193	7.9	21.2	3.8	5.4	0.48	0.25
194	12.2	19.5	5.8	6.7	0.48	0.34
195	8.5	20.5	4.2	5.7	0.49	0.28
196	12.2	22.8	5.0	5.8	0.41	0.25
197	5.2	18.6	4.0	4.6	0.77	0.25
199	13.0	21.7	5.2	6.4	0.40	0.29
200	7.1	18.6	4.4	4.7	0.62	0.25
201	25.9	30.5	9.3	10.1	0.36	0.33
202	11.2	18.9	5.4	6.5	0.48	0.34
203	9.3	19.8	4.1	6.1	0.44	0.31
204	4.6	19.6	2.6	4.1	0.57	0.21

Table 22. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*

Intravenous medication. Vaccinated, meningitic rabbits.

Rabbit No.	In Plasma		In Cerebrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total
207	27.4	29.4	9.6	19.3	0.35	0.66
208	26.0	29.5	15.0	18.8	0.58	0.64
209	19.5	27.7	15.9	19.6	0.82	0.71
210	20.9	25.0	11.5	13.5	0.55	0.54
214	28.0	30.1	12.4	13.3	0.44	0.44
215	24.9	28.0	11.1	12.1	0.44	0.43
216	21.2	25.9	8.1	9.4	0.38	0.36
217	25.9	27.8	12.0	13.4	0.46	0.48
218	25.9	28.5	11.6	12.5	0.45	0.44
219	25.9	28.5	16.4	18.4	0.63	0.65
220	20.9	28.0	11.5	15.0	0.55	0.54
221	16.5	22.8	9.1	12.0	0.55	0.53

Table 23. *Tabulation of the distribution of sulfapyridine in cerebrospinal fluid in non-infected and infected rabbits respectively.*

Means of the ratios between the concentration of sulfapyridine in cerebrospinal fluid and in plasma.

Intravenous medication.

Non-infected rabbits		Meningitic rabbits		Septic rabbits	
Free	Total	Free	Total	Free	Total
0.37 ± 0.016	0.31 ± 0.012	0.55 ± 0.023	0.46 ± 0.022	0.54 ± 0.021	0.40 ± 0.017
Difference as against non-infected rabbits		0.18 ± 0.028	0.15 ± 0.025	0.17 ± 0.026	0.09 ± 0.021

Non-infected rabbits		Vaccinated rabbits not infected		Vaccinated meningitic rabbits	
Free	Total	Free	Total	Free	Total
0.37 ± 0.016	0.31 ± 0.012	0.45 ± 0.034	0.29 ± 0.016	0.52 ± 0.037	0.54 ± 0.032
Difference as against non-infected rabbits		0.08 ± 0.038	−0.02 ± 0.020	0.15 ± 0.040	0.23 ± 0.034

Intramuscular medication.

Non-infected rabbits		Meningitic rabbits	
Free	Total	Free	Total
0.23 ± 0.017	0.22 ± 0.019	0.36 ± 0.017	0.37 ± 0.024
Difference as against non-infected rabbits		0.13 ± 0.024	0.15 ± 0.031

Subcutaneous medication.

0.35 ± 0.019	0.24 ± 0.014	0.46 ± 0.038	0.32 ± 0.044
Difference as against non-infected rabbits		0.11 ± 0.043	0.08 ± 0.046

for free Sp. was 0.54 ± 0.021, for vaccinated but not infected animals 0.45 ± 0.034, and for vaccinated meningitic animals 0.52 ± 0.037. Corresponding values for total Sp. were 0.40 ± 0.017, 0.29 ± 0.16 and 0.54 ± 0.032.

Table 24. *Distribution of Sulfapyridine between Tissue and Plasma.*

Intravenous medication. Meningitic rabbits.

Rabbit No.	In Plasma Total mg per 100 ml	In Brain Total mg per 100 gm	Ratio Brain value / Plasma value	In Muscle Total mg per 100 gm	Ratio Muscle value / Plasma value	In Liver Total mg per 100 gm	Ratio Liver value / Plasma value	In Spleen Total mg per 100 gm	Ratio Spleen value / Plasma value	In Lung Total mg per 100 gm	Ratio Lung value / Plasma value
97	25.7	16.7	0.65	24.2	0.94	27.5	1.07	23.1	0.90	28.3	1.10
98	24.0	12.7	0.53	20.1	0.84	24.0	1.00	20.6	0.86	26.7	1.11
99	36.3	22.0	0.61			30.6	0.84	28.3	0.78	31.2	0.86
100	26.0	16.6	0.64	24.6	0.95	26.6	1.02	24.0	0.92	26.5	1.02
101	23.6	10.7	0.45	18.7	0.79	26.1	1.11	21.2	0.90	25.5	1.08
102	28.0	18.6	0.66	25.1	0.90	29.2	1.04	25.1	0.90	28.5	1.02
103	33.1	20.0	0.60			28.1	0.85	26.2	0.79	28.3	0.85
104	32.1	19.6	0.61	28.3	0.88	30.6	0.95	27.2	0.85	30.6	0.95
105	26.7	13.3	0.50	18.5	0.69	20.6	0.77	19.0	0.71	24.0	0.90
106	36.7	24.0	0.65	32.2	0.88	32.2	0.88	27.5	0.75	32.2	0.88
107	34.7	18.7	0.54			30.0	0.86	27.6	0.80	28.8	0.83
108	48.6	21.6	0.44			32.2	0.66	27.6	0.57	30.0	0.62
109	30.0	23.7	0.79			32.2	1.07	28.8	0.96	32.4	1.08
110	26.7	15.7	0.59	21.8	0.82	27.2	1.02	32.8	1.23	26.2	0.98
111	21.7	11.0	0.51	16.3	0.75	21.8	1.00	15.7	0.72	21.8	1.00
114	32.7	17.5	0.54	26.2	0.80	27.8	0.85	21.8	0.67	27.2	0.83
115	21.2	10.1	0.48	13.5	0.64	19.6	0.92	14.2	0.67	18.5	0.87
116	21.5	12.7	0.59	15.4	0.72	21.6	1.00	19.6	0.91	21.2	0.99
117	28.1	15.1	0.53	19.2	0.68	26.2	0.92	21.2	0.75	27.2	0.96
118	23.8	15.0	0.63	18.0	0.76	21.9	0.92	20.5	0.86	25.2	1.06
119	22.8	12.7	0.56	17.1	0.75	19.8	0.87	18.0	0.79	22.9	1.01
120	24.6	16.0	0.65	21.8	0.89	25.6	1.04	22.3	0.91	25.2	1.02
121	22.3	15.7	0.70	17.8	0.80	23.8	1.07	20.8	0.93	24.0	1.08
122	21.9	16.8	0.77	20.0	0.91	24.0	1.10	20.5	0.94	24.0	1.10
123	22.7	12.4	0.55	18.0	0.79	21.8	0.96	18.0	0.79	22.0	0.97
124	20.2	14.4	0.71	16.5	0.82	22.1	1.10	18.5	0.92	22.0	1.09
125	23.3	16.8	0.72	20.6	0.88	24.0	1.03	22.2	0.95	24.2	1.04

Summary.—It is seen from the tables that infected animals receiving Sp. intravenously show a cerebrospinal fluid concentration of the free drug which is rather more than 50 per cent of that in the plasma at the times the samples were taken, while the fluid concentration in animals to which the drug was administered by the intramuscular and subcutaneous routes is somewhat less.

A study of table 17 shows the following from a statistical viewpoint: In theory, the lowest value should be down at 0.21 and the highest value up at 0.87. The empirical limit values are 0.40 and 0.84 respectively. In other words, they lie within the limits of the calculated range of variation. In view of the fact

Table 25. *Distribution of Sulfapyridine between Tissue and Plasma.*

Intramuscular medication. Meningitic rabbits.

Rabbit No.	In Plasma Total mg per 100 ml	In Brain Total mg per 100 gm	Ratio Brain value / Plasma value	In Muscle Total mg per 100 gm	Ratio Muscle value / Plasma value	In Liver Total mg per 100 gm	Ratio Liver value / Plasma value	In Spleen Total mg per 100 gm	Ratio Spleen value / Plasma value	In Lung Total mg per 100 gm	Ratio Lung value / Plasma value
130	14.1	4.8	0.34	6.8	0.48	9.3	0.66	8.3	0.59	11.4	0.81
131	12.9	3.1	0.24	7.2	0.56	8.7	0.68	8.0	0.62	12.0	0.93
132	11.5	4.3	0.37	6.3	0.55	8.0	0.70	6.3	0.55	14.7	1.28
134	24.0	14.5	0.60	20.9	0.87	24.4	1.02	11.9	0.50	26.5	1.10
135	12.8	4.6	0.36	6.7	0.52	11.6	0.91	9.6	0.75	13.4	1.05
136	22.2	10.6	0.48	15.3	0.69	21.6	0.98	21.5	0.97	22.0	0.99
137	10.9	5.3	0.48	7.3	0.67	9.7	0.89	10.5	0.97	10.5	0.97
138	15.3	3.8	0.25	6.3	0.41	11.5	0.75	8.6	0.56	12.4	0.81
139	16.3	6.3	0.39	9.8	0.60	14.5	0.89	12.8	0.79	17.5	1.07
140	14.0	5.1	0.36	6.7	0.48	12.2	0.87	9.8	0.70	14.2	1.01
141	16.0	4.9	0.30	9.7	0.61	19.0	1.19	11.8	0.74	14.7	0.92
142	17.2	8.8	0.51	11.3	0.66	16.7	0.97	13.0	0.76	19.3	1.12
143	13.3	5.9	0.45	8.6	0.65	13.1	0.99	9.5	0.72	14.6	1.10
144	14.2	6.0	0.42	7.2	0.51	9.5	0.67	9.0	0.63	13.9	0.98

that the material is small, it seems most natural to attribute to factors of chance the fact that the empirical range of variation is not symmetrical within the calculated one: There should hardly be any reason to expect the distribution to be skew, even if a slight skewness cannot be entirely excluded, which only becomes perceptible in a larger material. A possible skewness of this kind cannot, then, have any importance for the problems presented here.

Most of the other series in this investigation show no skew distribution.

To facilitate an idea of the difference in the values of healthy and infected animals, the mean ratios of the concentration in fluid and in plasma in all series have been drawn up in table 23, where the computed differences are also given. The table shows that, after intravenous injection, the mean ratio of the *concentration of Sp. in cerebrospinal fluid* and in plasma *in the infected animals has a higher value than in healthy animals,* so that a statistically significant difference is obtained for both free and total Sp.

The number of samples from the animals given Sp. intramus-

Table 26. *Distribution of Sulfapyridine between Tissue and Plasma.*

Subcutaneous medication. Meningitic rabbits.

Rabbit No.	In Plasma Total mg per 100 ml	In Brain Total mg per 100 gm	Ratio Brain value / Plasma value	In Muscle Total mg per 100 gm	Ratio Muscle value / Plasma value	In Liver Total mg per 100 gm	Ratio Liver value Plasma value	In Spleen Total gm per 100 gm	Ratio Spleen value / Plasma value	In Lung Total gm per 100 gm	Ratio Lung value Plasma value
145	10.1	1.8	0.18	4.4	0.44	5.0	0.50	4.6	0.46	6.5	0.64
146	23.3	4.4	0.19	8.4	0.36	14.6	0.63	11.3	0.48	14.0	0.60
147	26.1	10.3	0.40	17.5	0.67	21.8	0.84	16.3	0.62	24.0	0.92
148	23.8	7.3	0.30	13.5	0.57			16.8	0.71	20.7	0.87
149	26.9	7.4	0.28	12.9	0.48	19.8	0.74	16.0	0.59	21.9	0.81
150	23.6	9.5	0.40	14.6	0.62	19.6	0.83	17.1	0.72	22.0	0.93
151	28.0	12.1	0.43	19.6	0.70	25.1	0.90	22.9	0.82	30.6	1.09
152	18.9	5.5	0.29	8.6	0.46	13.5	0.71	10.3	0.54	13.7	0.72
153	15.3	5.7	0.37	10.5	0.69	13.2	0.86	13.5	0.88	20.7	1.35
154	10.3	3.7	0.36	6.8	0.66	9.1	0.89	6.8	0.66	10.5	1.02
155	11.8	5.5	0.47	6.5	0.55	7.8	0.66	10.2	0.86	12.5	1.06
156	19.7	5.0	0.25	9.5	0.48	16.8	0.85	13.1	0.66	11.7	0.59
157	30.1	20.6	0.69	27.2	0.91	30.6	1.02	31.1	1.03	28.3	0.94
158	28.0	10.5	0.38	19.0	0.68	27.2	0.97	20.1	0.72	25.1	0.90
159	34.2	16.6	0.49	28.3	0.83	32.8	0.96	32.8	0.96	33.9	0.99

cularly is small, so that it must be taken into account that the standard error obtained in the usual way may not give significant differences at 3 times the standard error limit. As, however, the difference amounts to about 5 times the standard error, and the number of animals is 11, the material can be considered large enough to make the differences significant. This does not apply to the series of animals that were injected subcutaneously.

Concentration of Sp. in Tissue.

Tables 24—29 show the concentration of total Sp. in plasma, and also its concentration in the respective organs. The ratios between the concentration in the different organs and in the plasma are also given.

For this determination the same animals were used as were examined earlier to decide the concentration in the blood and cerebrospinal fluid. The number of infected animals investigated after different administration of Sp. is shown in the tables. The mean ratios of the concentration in tissue and in plasma after *intravenous* injection of m e n i n g i t i c animals were, for

Table 27. *Distribution of Sulfapyridine between Tissue and Plasma.*

Intravenous medication. Septic rabbits.

Rabbit No.	In Plasma Total mg per 100 ml	In Brain Total mg per 100 gm	Ratio Brain value / Plasma value	In Muscle Total mg per 100 gm	Ratio Muscle value / Plasma value	In Liver Total gm per 100 gm	Ratio Liver value / Plasma value	In Spleen Total mg per 100 gm	Ratio Spleen value / Plasma value	In Lung Total mg per 100 gm	Ratio Lung value / Plasma value
164	19.3	6.8	0.35	13.0	0.67	17.8	0.92	17.4	0.90	20.1	1.04
165	21.7	10.2	0.47	17.4	0.80	20.1	0.93	16.3	0.75	30.6	1.41
166	19.7	13.9	0.71	22.9	1.16	24.0	1.22	22.7	1.15	24.0	1.22
167	17.0	9.8	0.58	15.5	0.91	16.8	0.99	13.8	0.81	17.3	1.02
168	24.0	11.4	0.48	20.0	0.83	25.0	1.04	21.8	0.91	27.3	1.14
169	29.9	18.5	0.62	27.8	0.93	30.6	1.02	27.3	0.91	31.2	1.04
170	25.4	13.4	0.52	21.2	0.83	26.1	1.03	22.8	0.90	25.7	1.01
171	14.0	6.3	0.45	9.6	0.69	14.7	1.05	10.2	0.73	15.7	1.12
172	21.6	9.4	0.43	15.4	0.71	24.0	1.11	16.4	0.76	21.8	1.01
173	24.3	10.5	0.43	18.6	0.77	26.1	1.07	17.6	0.72	25.7	1.06
174	30.0	17.8	0.59	28.3	0.94	31.7	1.06	27.0	0.90	32.8	1.09
175	24.6	16.7	0.68	21.8	0.89	26.2	1.07	24.0	0.98	28.3	1.15
176	18.4	9.7	0.52	14.6	0.79	16.7	0.91	13.5	0.73	18.7	1.02
177	20.7	9.0	0.43	13.5	0.65	17.4	0.84	15.0	0.72	20.7	1.00
178	23.8	15.3	0.64	22.7	0.95	26.4	1.11	20.9	0.88	25.0	1.05
179	27.5	15.5	0.56	22.2	0.81	27.3	0.99	22.0	0.80	28.3	1.03
180	21.8	12.3	0.56	16.9	0.78	20.5	0.94	17.6	0.81	20.5	0.94
181	23.9	13.5	0.56	21.3	0.89	24.2	1.01	19.8	0.83	24.2	1.01
182	22.5	12.9	0.57	19.8	0.88	28.3	1.26	20.0	0.89	26.5	1.18
183	25.0	13.7	0.55	20.9	0.84	26.2	1.05	22.4	0.90	27.8	1.12
184	22.3	14.7	0.66	22.0	0.99	24.6	1.10	23.1	1.04	24.0	1.08
185	22.1	13.0	0.59	18.8	0.85	22.8	1.03	19.6	0.89	23.8	1.08

liver and lung 0.96 ± 0.021 and 0.97 ± 0.020 respectively, for musculature and spleen 0.81 ± 0.018 and 0.84 ± 0.025 respectively, and for brain 0.60 ± 0.018. Corresponding values after *intramuscular* injection were 0.87 ± 0.042, 1.01 ± 0.033, 0.59 ± 0.031, 0.70 ± 0.038 and 0.40 ± 0.029 and after *subcutaneous* injection 0.76 ± 0.042, 0.90 ± 0.052, 0.61 ± 0.039, 0.71 ± 0.044 and 0.37 ± 0.033.

For the s e p t i c animals the mean ratios of the concentration in tissue and plasma were, for liver and lung 1.03 ± 0.021 and 1.08 ± 0.021 respectively, for musculature and spleen 0.84 ± 0.025 and 0.86 ± 0.023 respectively, and for brain 0.54 ± 0.020; for v a c c i n a t e d n o t i n f e c t e d animals the corresponding values were, for liver 0.86 ± 0.032, for musculature 0.63 ± 0.035, and for brain 0.41 ± 0.025; for v a c c i n a t e d m e n i n g i t i c animals the corresponding values were, for liver and lung 0.99 ±

Table 28. *Distribution of Sulfapyridine between Tissue and Plasma.*

Intravenous medication. Vaccinated rabbits, not infected.

Rabbit No.	In Plasma Total mg per 100 ml	In Brain Total mg per 100 gm	Ratio Brain value / Plasma value	In Muscle Total mg per 100 gm	Ratio Muscle value / Plasma value	In Liver Total mg per 100 gm	Ratio Liver value / Plasma value	In Spleen Total mg per 100 gm	Ratio Spleen value / Plasma value	In Lung Total mg per 100 gm	Ratio Lung value / Plasma value
188	22.5	13.0	0.58	22.5	1.00	26.7	1.19	20.5	0.91	24.2	1.08
189	26.0	10.9	0.42	17.4	0.67	23.5	0.90	18.1	0.70	23.3	0.90
190	22.7	6.4	0.28	12.0	0.53	19.6	0.86	15.2	0.67	20.2	0.89
191	21.1	11.0	0.52	15.5	0.73	21.9	1.04	17.0	0.81	22.2	1.05
192	25.9	14.2	0.55	20.1	0.78	23.4	0.90	19.6	0.76	26.2	1.01
193	21.2	6.0	0.28	9.6	0.45	13.9	0.66				
194	19.5	8.5	0.44	12.6	0.65	17.6	0.91				
195	20.5	6.8	0.33	10.8	0.53	15.5	0.76				
196	22.8	8.0	0.35	13.4	0.59	19.1	0.84				
197	18.6	6.3	0.34	10.0	0.54	16.8	0.90				
199	21.7	8.6	0.40	13.0	0.60	17.6	0.81				
200	18.6	7.8	0.42	10.0	0.54	15.0	0.81				
201	30.5	16.8	0.55	24.0	0.79	28.9	0.95				
202	18.9	7.6	0.40	10.5	0.56	14.6	0.77				
203	19.8	7.7	0.39	10.5	0.53	14.5	0.73				
204	19.6	5.2	0.26	10.7	0.55	15.3	0.78				

Table 29. *Distribution of Sulfapyridine between Tissue and Plasma.*

Intravenous medication. Vaccinated, meningitic rabbits.

Rabbit No.	In Plasma Total mg per 100 ml	In Brain Total mg per 100 gm	Ratio Brain value / Plasma value	In Muscle Total mg per 100 gm	Ratio Muscle value / Plasma value	In Liver Total mg per 100 gm	Ratio Liver value / Plasma value	In Spleen Total mg per 100 gm	Ratio Spleen value / Plasma value	In Lung Total mg per 100 gm	Ratio Lung value / Plasma value
205	25.8	16.5	0.64	21.2	0.82	27.8	1.08	22.6	0.88	27.5	1.07
206	28.0	21.2	0.76	26.3	0.94	28.3	1.01	29.7	1.06	32.5	1.16
207	29.4	27.4	0.93	29.3	1.00	34.0	1.16	30.2	1.03	41.0	1.39
208	29.5	22.7	0.77	24.0	0.81	30.0	1.02	25.0	0.85	27.4	0.93
209	27.7	19.0	0.68	25.3	0.91	28.5	1.03	24.8	0.89	29.7	1.07
210	25.0	19.1	0.76	25.0	1.00	26.1	1.04	25.5	1.02	30.5	1.22
211	26.1	16.7	0.64	23.8	0.91	27.2	1.04	24.0	0.92	27.9	1.07
212	39.0	16.6	0.43	26.2	0.67	31.2	0.80	27.5	0.71	30.6	0.78
213	26.4	13.6	0.51	20.6	0.78	27.1	1.03	20.5	0.78	24.0	0.91
214	30.1	23.6	0.79	27.2	0.91	28.3	0.94	24.8	0.83	30.3	1.00
215	28.0	16.3	0.58	20.2	0.72	28.3	1.01	22.7	0.81	27.0	0.96
216	25.9	14.1	0.54	21.5	0.83	23.4	0.90	21.8	0.84	26.2	1.01
217	27.8	19.3	0.70	21.8	0.79	27.2	0.98	24.0	0.86	26.2	0.94
218	28.5	18.4	0.64	26.2	0.92	30.6	1.07	28.3	0.99	30.6	1.07
219	28.5	20.6	0.72	26.2	0.92	30.1	1.06	27.2	0.95	29.5	1.04
220	28.0	15.9	0.57	18.1	0.65	22.7	0.81	21.0	0.75	24.0	0.86
221	22.8	12.0	0.52	14.2	0.62	18.6	0.82	15.8	0.69	24.0	1.05

Table 30. *Tabulation of the distribution of sulfapyridine in tissue in non-infected and infected rabbits respectively. Means of the ratios between the concentration of sulfapyridine in tissue and in plasma.*

Intravenous medication.

	Brain	Muscle	Liver	Spleen	Lung
Non-infected rabbits..	0.39 ± 0.015	0.63 ± 0.027	0.87 ± 0.030	0.65 ± 0.029	0.89 ± 0.026
Meningitic rabbits ...	0.60 ± 0.018	0.81 ± 0.018	0.96 ± 0,021	0.84 ± 0.025	0.97 ± 0.020
Difference as against non-infected rabbits	0.21 ± 0.023	0.18 ± 0.032	0.09 ± 0.037	0.19 ± 0.038	0.08 ± 0.033
Increase in per cent as against non-infected rabbits	54 %	29 %		29 %	
Septic rabbits	0.54 ± 0.020	0.84 ± 0.025	1.03 ± 0.021	0.86 ± 0.023	1.08 ± 0.021
Difference as against non-infected rabbits	0.15 ± 0.025	0.21 ± 0.037	0.16 ± 0.037	0.21 ± 0.037	0.19 ± 0.033
Increase in per cent as against non-infected rabbits	38 %	33 %	18 %	32 %	21 %
Vaccinated rabbits not infected.	0.41 ± 0.025	0.63 ± 0.035	0.86 ± 0.032		
Vaccinated meningitic rabbits	0.66 ± 0.030	0.84 ± 0.028	0.99 ± 0.025	0.87 ± 0.027	1.03 ± 0.034
Difference as against non-infected rabbits	0.27 ± 0.034	0.21 ± 0.039	0.12 ± 0.039	0.22 ± 0.040	0.14 ± 0.043
Increase in per cent as against non-infected rabbits	69 %	33 %	14 %	34 %	16 %

Intramuscular medication.

Non-infected rabbits..	0.32 ± 0.028	0.60 ± 0.032	0.96 ± 0.044	0.68 ± 0.045	1.04 ± 0.038
Meningitic rabbits....	0.40 ± 0.029	0.59 ± 0.031	0.87 ± 0.042	0.70 ± 0.038	1.01 ± 0.033
Difference as against non-infected rabbits.	+ 0.08 ± 0.040	− 0.01 ± 0.045	− 0.09 ± 0.061	+ 0.02 ± 0.059	− 0.03 ± 0.050

Subcutaneous medication.

Non-infected rabbits..	0.30 ± 0.021		0.87 ± 0.025	0.66 ± 0.023	0.94 ± 0.034
Meningitic rabbits....	0.37 ± 0.033	0.61 ± 0.039	0.76 ± 0.042	0.71 ± 0.044	0.90 ± 0.052
Difference as against non-infected rabbits	+ 0.07 ± 0.039		− 0.11 ± 0.049	+ 0.05 ± 0.050	− 0.04 ± 0.062

0.025 and 1.03 ± 0.034 respectively, for musculature and spleen 0.84 ± 0.028 and 0.87 ± 0.027 respectively, and for brain 0.66 ± 0.030.

As in the healthy animals, the concentration in the brain of the infected animals is low in relation to that in other organs. Of these, liver and lung have a high concentration, approaching that of the plasma, while that of musculature and spleen is lower. The concentration in the tissue is, throughout, higher after intravenous injection than after intramuscular and subcutaneous.

Summary.—The means for the ratios have been assembled in table 30, in which the corresponding values in healthy animals and the differences are also given. The table shows further the percentual increase for the different tissue ratios.

After *intravenous* injection the ratio between *the concentration in tissue* and in plasma *of the infected animals is higher than in the healthy ones. The increase is greatest for the brain.* The change in the tissue concentration is less marked after intramuscular and subcutaneous injection than after intravenous.

The former series do not give a significant difference between healthy and infected animals. If these two series are made into one a difference is obtained as regards the ratio between the Sp. concentration in brain and plasma which is very probable, although not significant.

This can be explained by the fact that the values of plasma and tissue concentration are lower after subcutaneous and intramuscular injection of the drug than after intravenous, as also that the investigated series are small. The different degree of acetylation in the different series may also be of importance in this respect.

Chapter 8.

The Importance of the Size of the Dose and different Modes of Administration.

The Importance of the Size of the Dose.

Both the healthy and the infected animals received the same dose of Sp. by intramuscular and subcutaneous injection. The dose given intravenously was 0.2 gm of Sp. per kg body-weight for the first 15 healthy animals, the first 18 meningitic animals, all the septic animals and all the vaccinated meningitic animals. All the other animals receiving the drug intravenously were given 0.1 gm per kg body-weight. To study how the size of the dose effects the distribution of Sp. in the blood, cerebrospinal fluid and tissue, comparisons have been made between the animals receiving 0.1 gm Sp. per kg body-weight and those receiving 0.2 gm Sp. per kg body-weight in the different series. It then transpired that both dosages gave a significant difference between the infected and the healthy animals. On the other hand, a comparison between this distribution in the healthy animals receiving the larger and the smaller dose respectively showed no significant difference. The same is true of the infected animals.

As there is *no difference in the distribution of Sp.* in blood, cerebrospinal fluid and tissue *attributable to the size of the dose,* the mean ratios in question from all the animals have been used when calculating means and standard errors in the different series.

The Influence of Different Modes of Administration.

The diagrams of the concentration of Sp. in plasma, cerebrospinal fluid and tissue at the time immediately following injection vary according to different modes of administering the drug. The diagrams also show that the plasma concentration of Sp. following intravenous injection sinks rapidly at the time for taking the samples, whereas that following intramuscular and subcutaneous injection reaches its highest value at this time.

The increase of the Sp. concentration in the cerebrospinal fluid and brain in infected cases is most marked after intravenous injection. The concentration on other organs follow, in the main, that of the plasma and is therefore on the down grade at the point of time in question after intravenous injection, whereas the one following intramuscular and subcutaneous injection reaches its highest value at this juncture.

The tables 1—30 show that the actual concentration values of Sp. in blood, cerebrospinal fluid and tissue are generally higher after intravenous injection than after the other two modes of administration. Similarly, the increase of the computed mean ratios in infected cases is higher after intravenous injection.

Thus, it can be seen from both diagrams and difference tables that the *increase of the concentration in corpuscles, cerebrospinal fluid and tissue of the infected animals is most marked after intravenous injection of Sp.*

Chapter 9.

Investigation into the Distribution of Sp. in the Organism of Rabbits before Infection and during its Subsequent Phases.

The following experiment was made to investigate the distribution of Sp. in the same specimens before, during and after a sustained infection: In a series of 6 animals the distribution of Sp. in whole blood, plasma and cerebrospinal fluid was first investigated in the usual way. After 2 days the animals were infected intracutaneously, according to the method prescribed, to induce sepsis. 2 days afterwards the animals were showing incipient symptoms of sepsis and had positive blood cultures. At this juncture parenteral Sp. therapy was instituted. 3 of the animals were given 0.2 gm of Sp. per kg body-weight subcutaneously twice a day; the remaining 3 received 0.1 gm pr kg body-weight intravenously 2 times a day. The treatment was continued for 4 days. One of the animals treated intravenously died after 3 days, and one of those treated subcutaneously died

after 5 days. 2 of the animals, one treated intravenously and one subcutaneously, were killed after 4 days, for a determination of the concentration in the organs. The remaining animals survived. The concentration of Sp. in blood and spinal fluid was determined daily in all the animals during the treatment. Determinations were also carried out on the survivors 3 times after recovery, at the usual time after the injection of the drug. The animals were controlled during the treatment for temperature, sedimentation rate and blood culture, as also agglutination titer in the blood. Diagrams have been made of these matters for the two surviving animals, fig. 21—22. Blood samples taken two days after the beginning of the treatment showed negative cultures in all the animals.

The concentration of Sp. in whole blood, plasma and cerebrospinal fluid was also determined in several healthy animals put on similarly continuous administration of Sp. Here the values varied very slightly. A diagram was made for the sake of comparison, fig. 23.

As can be seen from the diagrams, the ratio between the concentration of Sp. in corpuscles and in plasma of healthy animals is on a fairly constant level. In infected animals, on the other hand, the ratio value rises considerably at the height of the infection, afterwards gradually sinking. Fig. 22 shows a rising temperature on the 5th—7th days, corresponding to a new peak in the ratio curve. It is a moot point whether this indicates a toxic effect from the drug. In both cases the curves show the temperature to be already sinking when, after 4 days, the therapy is discontinued. The curve of sedimentation rate values show a rise similar to that of the curve for the ratio values during the height of the infection. The submitted values for the agglutination titer show that the antibodies begin to appear on the 7th day after infection set in, and reach their highest value after the 10th—11th days; this value they retain for some days, after which it sinks again.

The animals that died showed a similar rise of the ratio values, which had not yet dropped to normal before death.

The two animals who were killed showed a similar state of affairs in respect of the ratio between the concentration in cor-

Diagrams showing the distribution of Sp. in blood during continued treatment with Sp.

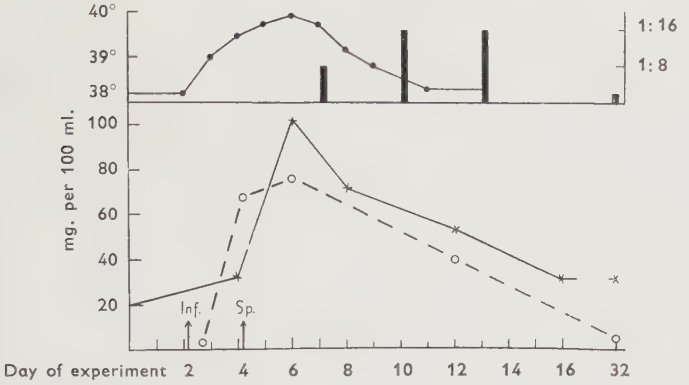

Fig. 21. Female septic rabbit during continued treatment with Sp.

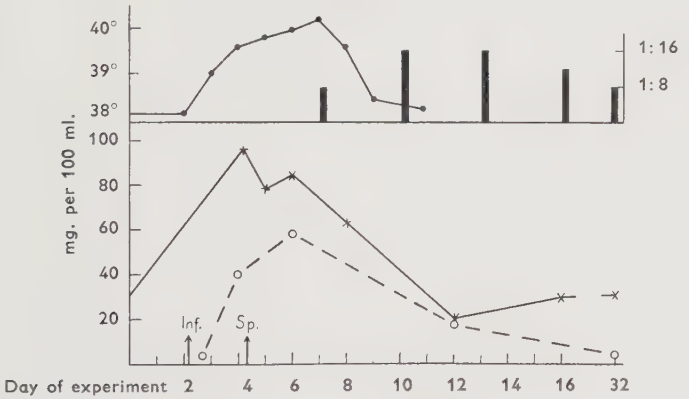

Fig. 22. Male septic rabbit during continued treatment with Sp.

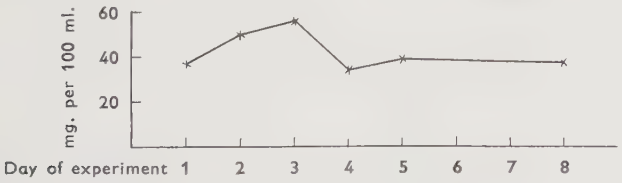

Fig. 23. Male non-infected rabbit during continued Sp.-experiment.

—x— the ratio of the concentr. of Sp. in corpuscles and plasma.
– – o – – S. R.
—•— temperature.
▌ agglutination-titre.

puscles and plasma. The relative concentration in the tissue was also raised, as was the case in the other infected animals, too.

Summary.—It appears from this investigation series that, *during the course of an infection, the concentration in corpuscles, cerebrospinal fluid and tissue rises in relation to the concomitant plasma concentration* in the same specimen. After the infection has subsided the values sink to what they were before its onset.

Chapter 10.

Acetylation.

The tables of the values for healthy and infected animals show how much of the free and the total drug is found in the blood corpuscles, plasma and cerebrospinal fluid in the different series, as has been discussed earlier on. As the total values constitute a sum of the free and the acetylated part, one cannot decide from them how much of the drug is present in conjugated form under different conditions. To study this more closely a selection has been made of the larger series of the animals receiving Sp. intravenously (tables 1, 10, 13). The free and total values in whole blood and plasma have been taken as a starting-point, and from these have been calculated the values for the concentration of free and acetylated Sp. in blood corpuscles and plasma. A comparison could subsequently be made between the concentration of acetylated Sp. in corpuscles and in plasma in healthy and infected animals respectively. It was found in so doing that no statistically significant difference could be obtained between the calculated means in this series. The remaining series are too small to be of use in assessing this question.

A similar procedure has been used to investigate the presence of acetylated Sp. in cerebrospinal fluid under different circumstances. The values for this have been calculated from the actual values for free and total Sp. in the fluid, in tables 4, 17, 20. The percentual amount of acetylated Sp. in the fluid (in relation to the total value) has been calculated for each specimen, after

Table 31 a. *The percentage of acetylated Sp. in the cerebrospinal fluid from healthy and infected animals, with calculated differences.*

Mean for the percent of acetylated Sp. in the fluid.

	Non-infected rabbits	Meningitic rabbits	Septic rabbits
	22 % ± 2.3 %	12 % ± 2.0 %	17 % ± 1.9 %
Difference as against non-infected rabbits.....		10 % ± 3.0 %	5 % ± 3.0 %

which the means for the different series and their standard error have been computed. The results from healthy and from meningitic and septic animals, all given Sp. intravenously, have been set out in table 31 a. The table shows that the percentage of acetylated Sp. in fluid from healthy animals is 22 % ± 2.3 %, whereas the corresponding value for meningitic animals is 12 % ± 2.0 % and for septic animals 17 % ± 1.9 %. As the calculated differences show, a statistically significant difference is obtained only between the two first-mentioned groups. It has been seen previously (p. 72) that both meningitic and septic animals show an increase in the ratios between the concentration of both free and total Sp. in fluid and plasma, as compared with healthy specimens. The above-mentioned circumstance that there is a lower percentage of the acetylated drug in the fluid from meningitic animals suggests that the previously observed relative increase in the Sp. concentration in the cerebrospinal fluid of such animals is largely due to an increase of the percentage of free drug in the fluid.

Table 31 b. *The percentage of acetylated Sp. in the plasma of healthy animals after different application of the drug, with calculated differences.*

Mean for the percent of acetylated Sp. in the plasma.

Intravenous medication	Subcutaneous medication	Intramuscular medication
43 % ± 2.8 %	54 % ± 2.8 %	42 % ± 4.4 %
Difference.....	11 % ± 4.0 %	

To investigate how different ways of applying the drug affect the degree of acetylation, the actual values for free and total Sp. in the plasma have been made the basis for the calculation of the percentage of acetylated Sp. (in relation to total Sp.) in the plasma of healthy animals after different modes of injection. The means in the different series are seen from table 31 b. This shows that the percentage of the drug in the plasma is higher after subcutaneous injection than after intravenous injection. The difference obtained is statistically probable, though not significant.

Summary.—The investigations into the presence of the acetylated part of Sp. in blood corpuscles and plasma thus show that *neither the healthy nor the infected animals display any tendency to accumulate the acetyl-Sp. in corpuscles or plasma.* The investigation of the same matter *in the cerebrospinal fluid* shows that *the acetylated part of the drug in the meningitic animals is lower than in the healthy* and the septic specimens.

The investigation into the effect of different administration of Sp. on the degree of acetylation in the plasma shows this *acetylation to be greater after subcutaneous injection of Sp. than after intravenous* or intramuscular injection.

Chapter 11.

Influence of Sex Factors.

The investigation into the distribution of Sp. in blood, cerebrospinal fluid and tissue has been made on a material of male animals, as mentioned before. To get an idea of the situation in females, similar investigations have been made on a series of 20 female, healthy specimens. Here the distribution in blood and fluid was studied. The values are shown in table 32. The plasma contained on an average 82 % $\pm$ 0.9 % free Sp. and 88 % $\pm$ 1.0 % total Sp., the corpuscles 18 % $\pm$ 0.9 % and 12 % $\pm$ 1.0 % respectively. The mean ratio of the concentration in corpuscles and in plasma was, for free Sp. 0.55 ± 0.030 and for total Sp. 0.34 ± 0.033. Corresponding values for males are shown in table 1.

Table 32. *Distribution of Sulfapyridine between Corpuscles and Plasma.*

Intravenous medication. Female rabbits, not infected.

Rabbit No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
225	4.3	13.2	4.9	16.5	69	79	86	31	21	14	0.59	0.36
226	6.1	11.4	6.8	13.6	74	82	88	26	18	12	0.63	0.39
227	5.3	11.8	6.1	14.3	70	81	85	30	19	15	0.55	0.41
228	5.8	11.0	7.1	14.0	72	88	92	28	12	8	0.35	0.22
229	3.5	12.1	3.7	14.9	71	75	87	29	25	13	0.81	0.36
230	4.1	13.8	4.6	18.2	72	81	95	28	19	5	0.60	0.14
231	8.7	14.3	9.5	17.4	69	75	84	31	25	16	0.74	0.42
232	3.0	10.0	3.5	14.1	70	82	99	30	18	1	0.51	0.02
233	8.3	12.6	9.7	15.4	70	82	86	30	18	14	0.51	0.38
234	9.2	20.2	11.0	24.0	69	83	82	31	20	14	0.58	0.38
235	5.4	12.7	6.1	16.0	71	80	89	29	20	11	0.61	0.30
236	2.9	13.1	3.2	16.2	70	77	87	30	23	13	0.70	0.35
237	6.6	14.0	8.2	19.6	65	81	91	35	19	9	0.44	0.18
238	5.4	13.1	6.2	14.0	75	86	80	25	14	20	0.49	0.75
239	9.2	12.8	10.1	15.6	71	78	87	29	22	13	0.69	0.36
240	11.4	17.8	14.3	23.2	70	88	91	30	12	9	0.32	0.23
241	11.2	15.1	12.8	18.5	70	80	86	30	17	18	0.46	0.49
242	11.6	18.5	14.7	23.5	70	89	89	30	11	11	0.29	0.29
243	14.7	18.8	16.2	21.8	69	76	80	31	24	20	0.70	0.56
244	14.0	18.0	16.5	22.0	70	83	86	30	17	14	0.48	0.38
245	10.6	18.0	12.3	22.0	75	87	92	25	13	8	0.45	0.26

Table 33. *Distribution of Sulfapyridine between Cerebrospinal Fluid and Plasma.*

Intravenous medication. Female rabbits, not infected.

Rabbit No.	In Plasma		In Cerebrospinal Fluid		Ratio Amount in 100 ml Cerebrospinal Fluid / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	Free	Total
234	11.0	24.0	4.0	6.3	0.36	0.26
235	6.1	16.0	4.3	4.9	0.70	0.31
236	3.2	16.2	2.6	3.5	0.81	0.22
237	8.2	19.6	4.5	6.2	0.55	0.32
238	6.2	14.0	3.0	5.0	0.48	0.36
239	10.1	15.6	3.6	4.3	0.36	0.28
240	14.3	23.2	5.5	7.0	0.38	0.30
241	12.8	18.5	6.1	6.5	0.48	0.35
243	16.2	21.8	7.0	8.0	0.43	0.37
244	16.5	22.0	4.8	6.5	0.29	0.30
245	12.3	22.0	5.1	11.4	0.41	0.52

As table 32 shows, only 10 samples of cerebrospinal fluid were obtained in this series. The mean ratio between the concentration of Sp. in fluid and in plasma was, for free Sp. 0.48 ± 0.047 and for total Sp. 0.33 ± 0.024.

To study the action of the infection on the distribution of Sp. in corpuscles, plasma and cerebrospinal fluid in female animals these matters were investigated in the same specimen before, during and after infection. These cases, too, showed a rise of the ratio values at the height of the infection. The diagram on p. 83 shows the course in one of these animals.

As the above values show, there is *a* very probable statistical *difference between healthy males and females as regards the distribution of Sp. in the blood,* although it is not significant. In the females investigated with reference to this the infection caused the same change of drug distribution as in the male animals.

Chapter 12.

Dialysis Experiments.

As has been pointed out earlier by others, part of the sulfanilamide derivatives in the blood is present in a form bound to the protein substances of the plasma. It has been established that the amount bound to the protein in this way is not the same for each derivative. Thus, Davis (22) submits the following figures, based on dialysis experiments for the amount of sulfanilamide derivatives which is bound to the plasma protein: Sulfanilamide 20 per cent, Sp. 40 per cent, Sulfathiazole 75 per cent.

The following dialysis experiment was made to discover if the *infection* and its attendant *changes* in the protein relations of the blood plasma affect the *adsorption of Sp.*

Serum from 6 healthy and 6 septic animals was examined.

Method.—Dialysis took place in a cellophane tube whose ends were plugged by introducing a rubber cork and then binding the tube round tight with a silk thread. It was then placed in a glass tube large enough for it to be moved backwards and forwards with ease. Four times as

Dialysis tests.

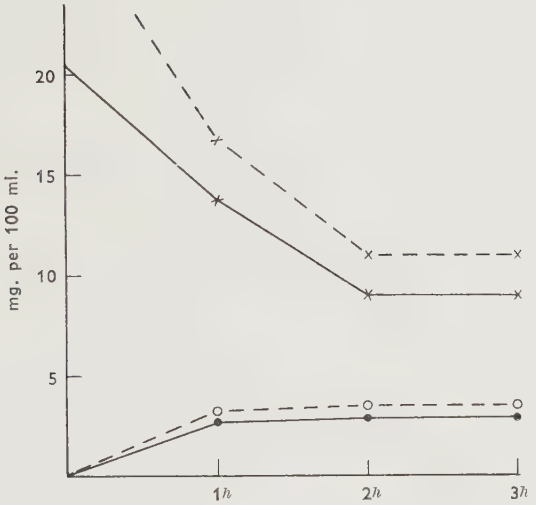

Fig. 24. Dialysis of serum from non-infected rabbit.

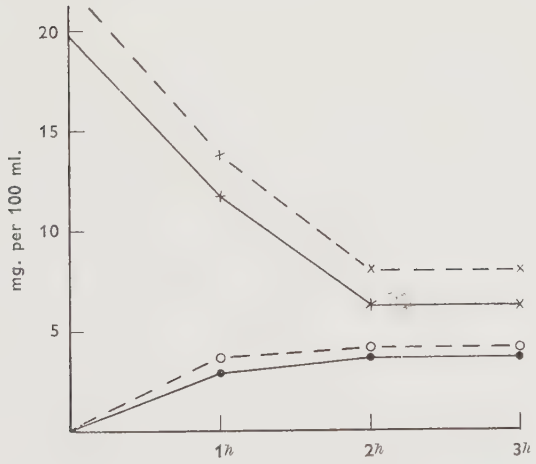

Fig. 25. Dialysis of serum from septic rabbit.

—x— concentr. of free Sp. in inner fluid.
– – x – – » » total Sp. » » »
—•— » » free Sp. » outer »
– – o – – » » total Sp. » » »

much outer fluid as plasma was poured in. The outer fluid consisted of a phosphate buffer whose p^H was about 7.7, added to a solution of sodium chlorid.[1] The tube was carefully corked and placed on a cradle, whereupon the cellophane capsule was impelled backwards and forwards in the outer fluid. After set intervals a small sample was taken from the outer fluid to determine the Sp. concentration. When constant values had been reached dialysis was discontinued and samples taken from both outer and inner fluids for determining the concentration. In some cases samples were also taken of the inner fluid during dialysis.

Diagrams were made from the values obtained. Two such diagrams are given above, fig. 24, 25.

In serum from healthy animals the dialyzable part of free Sp. formed 24—36 per cent of the inner fluid's concentration. The corresponding values for acetyl-Sp. was 15—28 per cent. In serum from infected animals the value for the dialyzable part of free Sp. was 56—74 per cent of the inner fluid's concentration. The corresponding values for acetyl-Sp. was 24—38 per cent.

It appears from these experiments that more Sp. occurs in a dialyzable form in serum from the septic animals than in serum from healthy animals. The relative values for the dialyzable part of acetyl-Sp. are lower than for the free Sp. in both healthy and infected animals.[2]

The number of animals investigated in this respect is too small to clinch the question of the change in the amount of dialyzable Sp. in serum from infected animals, as compared with healthy ones. A certain importance may nevertheless be accorded to the values elicited, since a marked agreement is obtained between the figures from the dialyzable part of Sp. in serum from different animals, and the differences between the values from infected and healthy animals is great.

[1] The composition of the fluid was such that the molarity of NaCl = 0.1, of Na_2HPO_4 = 0.016 and of NaH_2PO_4 = 0.002.

[2] The author has studied these conditions in electrophoretic experiments too. In these tests which are not yet concluded there was also a certain difference between the amount of sulfapyridine bound to the different protein fractions in serum from healthy and infected cases respectively.

Chapter 13.

Discussion of the Results of the Author's Experimental Investigations.

In *healthy animals* the relation between the concentration in blood corpuscles and in plasma for free Sp. is about 40 per cent, varying somewhat after different modes of administration. Hansen (52), who carried out similar experiments on rabbits, had, in a smaller series, a corresponding value of 42 per cent. These values are considerably lower than the one in man, indicating that the corpuscles of the healthy rabbit are less permeable to the drug than are those of man. The concentration of free Sp. in the cerebrospinal fluid of healthy rabbits is about 30—35 per cent of the plasma concentration, whereas the corresponding value in man is round about 60 per cent (42). Thus, the permeability of free Sp. to the cerebrospinal fluid, too, is apparently lower in the healthy rabbit than in healthy man. The concentration in tissue varies for the different organs investigated, but the values in the brain are considerably lower than in the other organs.

The *infected animals* show an increase in the permeability of Sp. into corpuscles, cerebrospinal fluid and tissue. This state of affairs tallies with the observations made on clinical material as to the passage of Sp. to cerebrospinal fluid in meningitis. On the other hand, investigation on clinical or experimental material as to the change in permeability of Sp. into blood corpuscles and tissue in infection has not been made before. Thus, there are no possibilities of comparison with other investigators.

Frisk (42) has observed a *parallel* between the passage of the different sulfanilamide derivatives to red corpuscles and to cerebrospinal fluid in the *healthy human subject.* The increase observed by me in permeability of Sp. to corpuscles *in* experimental *infection* in animals also runs parallel with a similar enhanced permeability to cerebrospinal fluid and tissue. This indicates a certain causal connection between the permeability of Sp. to corpuscles, cerebrospinal fluid and tissue in both healthy and infected animals. *A lesion changing the permeation at one place*

would consequently also change it at another. If this is confirmed on clinical material, it should be possible to conclude, from the increase of drug concentration in the corpuscles during an infectious state, what the approximate concentration is in cerebrospinal fluid and tissue in the individual in question. By determining the Sp. concentration in corpuscles and plasma it might be possible to assess the degree of the infectious toxic disturbances and the attendant general increase of permeability of Sp. in the infected subjects. Differences observed by me in the permeability of Sp. into blood corpuscles and cerebrospinal fluid before, during and after infection of the animals investigated serially indicate this to be the case in infected animals.

A very interesting thing is that the septic animals had negative fluid cultures and histologically no meningitis. Thus, although there was no infection in the meninges, as mentioned before, the passage of Sp. to the cerebrospinal fluid was increased. This points to an increase of the passage of Sp. in sepsis from blood to fluid which is about as pronounced as that in meningitis, even without a local process in the meninges. Similarly, I have shown that an increase of Sp.concentration, greater in the brain than in other tissue, can be observed in the experiment animals after septic infection, too, though less pronounced than after meningitis. *It would therefore seem that the toxic lesion which changes the permeability results from the general action on the blood vessels and is not directly due to a local process.* Yet a mainly local process may quite probably increase the local permeability for Sp. too; this may explain the observed greater increase in permeability for Sp. to the brain in meningitic rabbits as compared with septic rabbits.

When assessing the values of the tissue concentration, it must be taken into account that the tissue includes capillary blood and the lymph of the interstitial spaces. In this connection it may be mentioned that the volume of capillary blood in the human brain has been calculated at something more than one per cent of the brain weight (86). With regard to the brain the chemical stain reaction showed that *Sp. has an affinity for the gray substance, particularly its nerve-cells.* To illustrate this further analytical investigations of the Sp. concentration in only

the gray substance have been carried out in a few (six) cases: this gave appreciably higher values (about the double) than the corresponding investigation of white substance only from the same brain; other organs have not been studied in this respect. The author has here utilized the chance of *examining, in the brain, the Sp.-concentration in those different parts containing different quantities of certain tissue elements, namely the ganglia cells.* My histological findings indicate that the concentration in the brain is low compared with that of the other organs because only certain cellular elements there show greater affinity for Sp. The other organs show different concentrations of the drug. The relation between the concentration in the respective organs and in the plasma is nevertheless fairly constant in different individuals; it is therefore probable that the same causes hold for other organs as for the brain—i. e. that *an uneven distribution of Sp. in the different organs may be thought to exist, owing to varying affinity for Sp. in different tissue elements.*

When studying the tissue concentration diagrams one is struck by two things: firstly, that the brain concentration, as already mentioned, is so markedly low in relation to other organs; secondly, that the tissue concentration diagrams do not intersect those of the plasma. Intravenous injection in particular, where the plasma's fall in concentration is rapid, should enable the tissue concentration to exceed that in the plasma in certain phases. In point of fact, this sometimes happens among the infected animals, but it is in no wise a regular occurrence. However, these peculiarities in the diagrams can be explained by the above-mentioned supposition that only certain elements in the tissue take up the Sp.

The situation at the passage of the drugs to cerebrospinal fluid will be discussed first, to give an idea of the factors involved in the passage to tissue. Unlike tissue, cerebrospinal fluid is a physically homogenous substance; the above-mentioned irregularities in the distribution of the drug within different tissue elements will thus be eliminated.

The achievement of a certain concentration of a substance in cerebrospinal fluid is influenced by, in the main, one or more of the following factors.

1) If the substance in the blood occurs both *bound to the protein bodies* of the plasma and also free in the plasma, then perhaps only one of these forms is able to pass over to cerebrospinal fluid, namely the free form. The part passing over to the fluid would thus be made up of the dialysable part of the substance in the plasma.

2) If the biological boundary membrane separating blood and fluid has an *electrostatic countereffect,* then substances charged in a certain way can pass it only with difficulty. In this case the term "irreciprocal permeability" has been used; it implies that positively charged substances, for example, are able to pass easily, whereas negatively charged ones can only pass with difficulty.

3) If there is a permeable interstitial space between the blood and the cerebrospinal fluid, the *solubility of the substance* in question might be assumed to be *different* on the two sides of the interstitial space: avertine, for example, is more soluble in tissue rich in lipoids, e. g. brain, than in plasma (45).

4) Some form of *inactivation of the substance* in the cerebrospinal fluid may be thought to have taken place. This may be effected by utilization, combustion or other changes of the substance in the fluid. An adsorption of the substance to surrounding tissue, e. g. the brain and spinal cord, is also conceivable.

Of the possibilities presented here for the passage of a substance from blood to cerebrospinal fluid and the factors influencing the concentration in the latter, No. 1 seems to give the simplest explanation for the situation *as regards sulfanilamide derivatives.* The author's dialysis experiments show that, in healthy animals, the dialyzable Sp. constitutes, in the plasma, only a part of the drug. Davis (22) has shown, as already mentioned, that the proportion of the dialyzable part differs in different preparations. Andersen (1) has shown that the sulfathiazole in the cerebrospinal fluid from normal cases is wholly amenable to ultrafiltration, whereas a small part in that from a case of meningitis is not. The same author explains this by saying that the non-ultrafiltrable part of the sulfathiazole has accompanied the protein into the fluid.

A considerably *larger quantity of dialyzable Sp.* was revealed in dialysis of serum from *infected animals* in the author's experiments. This is interesting, because it can explain the increase of the concentration of Sp. in the cerebrospinal fluid of the *septic animals.* A similar increase in *meningitic animals* can, as Andersen showed, be explained partly by the concurrent protein increase in the fluid.

The other above-mentioned factors explaining the concentration of Sp. in cerebrospinal fluid cannot be left out of the question. One or more may conceivably apply simultaneously. Nevertheless, the agreement between the dialysis of serum containing Sp. from infected and healthy animals, and the values obtained for the permeability for Sp. to *cerebrospinal fluid* in healthy and infected animals respectively, suggests that the Sp.-concentration in the fluid depends in a certain measure on the amount of dialyzable drug in the serum.

The author has previously shown an agreement between the permeability of Sp. in cerebrospinal fluid and in corpuscles in both healthy and infected animals; it is therefore probable that the factors mentioned above for the permeability of Sp. to spinal fluid apply, to a certain extent, to the permeability of Sp. to *corpuscles* also.

The state of affairs in the *tissue is,* as has been mentioned previously, more complicated, both because the tissue is not a homogeneous substance, and on account of the different content of water, protein and other substances in different tissues. This makes it more difficult to evaluate the passage of Sp. from blood to tissue. The same factors as for cerebrospinal fluid might be thought to be present, however.

CLINICAL SECTION.

Chapter 14.

Distribution of Sulfathiazole between Corpuscles and Plasma in Man.

The distribution of sulfathiazole in blood corpuscles and plasma in infected man was investigated in blood samples from 39 patients in the oto-laryngologic clinic of Akademiska Sjukhuset. They were treated with sulfathiazole for acute infectious diseases and had enhanced sedimentation rate and generally fever. In 19 of these cases an exanthema had set in during the sulfathiazole treatment. Casuistic reports of these cases are available at the University Library of Upsala. For control purposes similar investigations were made of blood samples from 17 healthy individuals receiving sulfathiazole. 13 of the exanthema cases, 9 of the other infected cases and 9 of the healthy cases were females. All the cases had been on sulfathiazole therapy for at least 18 hours before the samples were taken. The exanthema cases had, in general, been receiving treatment for several days. A determination of the sulfathiazole concentration in whole blood and plasma, and also a haematocrite determination, were made on all blood samples. The values are seen in tables 34—36. They show that the concentration of free and total sulfathiazole alike is, in all cases, higher in the plasma than in the whole blood. In healthy individuals the plasma contained on an average 84 % ± 1.6 % of the free drug and 85 % ± 2.3 % of the total; the corpuscles contained 16 % ± 1.6 % and 15 % ± 2.3 % respectively. For the infected subjects the corresponding values are 80 % ± 1.4 % and 82 % ± 1.2 % in the plasma, and 20 % ± 1.4 % and 18 % ± 1.2 % in the corpuscles. The exanthema cases had lower values in the plasma: 73 % ± 1.7 % free substance and 75 % ± 1.7 % total substance, while the corpuscles contained 27 % ± 1.7 % and 25 % ± 1.7 % respectively. The mean of the ratios between the concentration in corpuscles and in plasma was, for

Table 34. *Distribution of Sulfathiazole between Corpuscles and Plasma.*

Oral medication. Infected cases.

Patient No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
1	5.6	7.1	6.7	9.5	62	74	83	38	26	17	0.57	0.33
2	4.5	5.4	6.4	7.0	55	78	71	45	22	29	0.34	0.50
3	6.3	8.0	8.4	10.6	61	81	81	39	19	19	0.37	0.37
4	3.1	4.2	4.0	5.3	62	80	78	38	20	22	0.41	0.46
5	5.6	7.1	6.8	9.1	63	77	81	37	23	19	0.51	0.40
6	4.8	4.9	6.2	6.7	63	81	86	37	19	14	0.40	0.28
7	6.5	7.8	8.5	11.1	65	85	93	35	15	7	0.33	0.14
8	6.0	7.4	8.5	10.8	55	78	80	45	22	20	0.34	0.31
9	5.6	6.5	7.9	9.0	59	83	82	41	17	18	0.29	0.32
10	5.4	6.9	7.6	9.2	63	89	84	37	11	16	0.21	0.32
11	7.9	8.1	8.7	9.2	63	69	72	37	31	28	0.76	0.66
12	3.5	4.1	4.8	5.4	63	86	83	37	14	17	0.28	0.35
13	5.0	6.2	6.9	8.3	64	88	86	36	12	14	0.24	0.29
14	2.2	3.4	2.5	4.3	62	70	78	38	30	22	0.70	0.46
15	4.7	6.4	4.8	7.4	71	73	82	29	27	18	0.91	0.54
16	5.4	6.6	7.5	8.5	65	90	84	35	10	16	0.21	0.35
17	7.3	8.5	8.9	11.0	59	72	76	41	28	24	0.56	0.45
18	6.9	7.2	7.8	8.6	70	79	84	30	21	16	0.62	0.44
19	5.1	6.3	6.7	9.2	63	83	92	37	17	8	0.35	0.15
20	9.3	10.0	12.1	12.8	67	87	86	33	13	14	0.30	0.33

free and total sulfathiazole respectively, 0.27 ± 0.031 and 0.25 ± 0.036 in healthy individuals, 0.44 ± 0.044 and 0.37 ± 0.027 in infected individuals, and 0.59 ± 0.044 and 0.54 ± 0.047 for the exanthema cases. For all the infected cases (incl. exanthema cases) the corresponding values were 0.51 ± 0.034 for the free and 0.46 ± 0.030 for the total substance.

The difference between healthy and infected subjects = 0.17 ± 0.054; 0.12 ± 0.045 and between healthy and exanthema cases = 0.32 ± 0.054; 0.29 ± 0.059 for free and total sulfathiazole respectively. Between healthy and all the infected subjects (incl. exanthema cases) the difference was 0.24 ± 0.046 for the free and 0.21 ± 0.047 for the total substance.

No sex difference was observed.

Table 35. *Distribution of Sulfathiazole between Corpuscles and Plasma.*

Oral medication. Exanthema cases.

Patient No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free mg per 100 ml	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
21	4.1	5.4	4.5	5.6	65	71	67	35	29	33	0.76	0.91
22	6.0	6.8	6.7	9.6	60	67	85	40	33	15	0.74	0.26
23	4.6	6.0	5.0	6.7	62	67	69	38	33	31	0.80	0.73
24	3.2	3.7	4.4	4.9	65	89	86	35	11	14	0.23	0.30
25	3.1	3.6	3.4	3.8	62	68	65	38	32	35	0.77	0.88
26	4.5	5.2	4.9	5.7	61	66	67	39	34	33	0.80	0.77
27	5.0	6.8	5.6	8.2	61	68	74	39	32	26	0.73	0.55
28	4.9	5.1	5.4	6.1	60	66	72	40	34	28	0.77	0.58
29	5.9	6.6	7.7	7.9	65	85	78	35	15	22	0.33	0.52
30	4.1	4.6	4.9	5.5	63	75	75	37	25	25	0.57	0.57
31	5.4	6.1	7.2	8.7	58	77	83	42	23	17	0.41	0.28
32	4.8	5.2	5.9	6.0	56	69	65	44	31	35	0.57	0.69
33	1.7	2.6	2.1	3.1	53	65	63	47	35	37	0.61	0.66
34	5.4	6.4	7.2	8.9	59	79	82	41	21	18	0.38	0.32
35	6.2	7.9	8.6	10.8	59	82	81	41	18	19	0.32	0.34
36	6.1	6.8	7.0	8.4	62	71	77	38	29	23	0.67	0.49
37	5.3	5.9	6.7	7.9	63	80	84	37	20	16	0.43	0.32
38	6.1	8.0	6.5	10.0	64	68	80	36	32	20	0.84	0.44
39	3.1	4.0	3.8	4.5	64	78	72	36	22	28	0.50	0.69

Table 36. *Distribution of Sulfathiazole between Corpuscles and Plasma.*

Oral medication. Non-infected cases.

Patient No.	In Whole Blood		In Plasma		Plasma Volumes	In Plasma		Corpuscles Volumes	In Corpuscles		Ratio Amount in 100 ml Corpuscles / Amount in 100 ml Plasma	
	Free mg per 100 mj	Total mg per 100 ml	Free mg per 100 ml	Total mg per 100 ml	%	Free %	Total %	%	Free %	Total %	Free	Total
1	5.9	6.5	8.5	9.0	56	81	78	44	19	22	0.30	0.36
2	4.6	5.4	6.6	7.4	56	80	77	44	20	23	0.32	0.38
3	5.9	7.0	8.1	9.7	58	80	80	42	20	20	0.35	0.35
4	5.6	6.0	9.3	9.7	56	93	91	44	7	9	0.10	0.13
5	7.6	8.0	9.9	10.0	56	73	70	44	27	30	0.47	0.54
6	5.8	7.3	8.0	10.0	63	87	86	37	13	14	0.25	0.28
7	6.8	8.1	10.0	11.6	57	84	82	43	16	18	0.25	0.29
8	5.4	6.1	7.9	8.7	50	73	71	50	27	29	0.37	0.41
9	5.3	6.4	8.2	10.2	60	93	96	40	7	4	0.11	0.06
10	6.3	8.3	9.1	12.0	60	87	87	40	13	13	0.22	0.22
11	5.3	7.1	8.6	10.9	50	81	77	50	19	23	0.23	0.30
12	3.6	4.0	4.9	6.0	62	84	93	38	16	7	0.31	0.12
13	7.3	8.4	11.4	13.6	60	94	97	40	6	3	0.10	0.05
14	5.9	6.5	9.2	11.0	58	90	98	42	10	2	0.15	0.03
15	9.2	10.2	12.7	14.7	60	83	86	40	17	14	0.31	0.24
16	9.1	9.6	10.6	12.6	70	82	92	30	18	8	0.51	0.20

Summary.

The investigation into the distribution of sulfathiazole in corpuscles and plasma in clinical material has thus shown the drug to appear in a considerably higher concentration in the latter. The ratio between the concentration in corpuscles and plasma respectively is higher in the infected subjects than in the healthy ones: this is particularly marked in the exanthema cases. The differences observed are statistically significant. This implies that there is an *increased permeability to corpuscles for sulfathiazole among the infected subjects.*

The material is too small for a definitive assessment either of the distribution of sulfathiazole in corpuscles and plasma or of the tendency to acetylate in infected and healthy subjects respectively. The noted increase of permeability of the corpuscles in the infected cases nevertheless indicates that the same conditions can be thought to rule the sulfathiazole treatment of infected human beings as the Sp. treatment of infected animals, previously shown. From this point of view special interest attaches to the fact that this *increase of permeability is particularly marked in the exanthema cases,* i. e. cases showing toxic manifestations.

Part III.

GENERAL DISCUSSION.

Chapter 15.

The Influence of Infection on the Distribution of Sulfanilamide Derivatives in the Organism.

In the study of the distribution of sulfanilamide derivatives within the organism, interest has previously concentrated mainly on the ways in which the different preparations differed from one another in this respect. These investigations were made largely on healthy subjects. Little study has, on the other hand, been devoted to the influence of infection on the distribution of sulfanilamide derivatives within the organism.

A number of the toxic manifestations observed in the treatment with sulfanilamide derivatives seem to indicate that the drugs become more toxic during infections. In the present investigations, therefore, the author has studied *inter alia* the distribution of sulfanilamide derivatives within the organism during infection and in connection with the appearance of certain toxic manifestations.

Experimental Section.

When assessing the influence of infection on the distribution of Sp. regard must be paid both to the factors deciding this distribution in healthy animals, and to the way in which infection acts on these factors.

The investigation into the distribution of Sp. in *healthy animals* has shown that the drug is distributed in a typical way in corpuscles, cerebrospinal fluid and tissue. Earlier investigators

have shown that this distribution has a certain connection with the extent to which the different drugs are bound to the plasma protein. The author's dialysis experiments also argue for this being the case, i. e. that the distribution of Sp. within the organism, and particularly in blood corpuscles and cerebrospinal fluid, depends to a certain extent on how great a part of the drug present in the plasma is dialyzable. As discussed in previous pages, other factors, too, may affect the distribution within the organism. Regarding the tissue, the distribution is probably irregular, certain elements having an affinity for Sp. while others have not. The piece of tissue used for determination of the concentration includes the capillary blood and the interstitial tissue fluid. These factors complicate the assessment of the concentration of Sp. in tissue under normal circumstances as well as after infection.

Now, what part does *infection* play in all this? The experiments with dialysis argue for an increase in the amount of dialyzable substance in serum from infected animals. It is not seen from the experiments carried out whether infection produces a change in the affinity of certain tissue elements for Sp., observed in healthy animals, though such a change would seem unlikely. An increase in the volume of the capillaries is thought to exist in grave infection; in the more pronounced conditions caused by infection when the collapse of the peripheral circulation sets in, there is thought to be an increase of the interstitial fluid also (10). On the other hand, certain compensating forces come into play at infection which attempt to readjust the tissue's content of interstitial fluid. A safety valve is represented here by the lymphatic systems, which has a great capacity for restoring interstitial fluid to the circulating blood (58). In my experimental investigations the animals were exposed to an extremely grave infection and showed signs of peripheral circulatory collapse, as mentioned previously, so that the conditions cited must be considered as holding for these specimens.

The influence of the infection on those factors which, under normal conditions, determine the distribution of Sp. in the organism, manifests itself in the *increased permeability* observed by

me for the drug to the blood corpuscles, cerebrospinal fluid and tissue of the infected animals. The increase of dialyzable Sp. in the serum seems the simplest explanation for the increased permeability of Sp. to corpuscles and cerebrospinal fluid. It can also be explained by the other factors in the infected animals mentioned earlier on. An increase of the amount of blood in the capillaries and interstitial fluid containing the drug may be thought to contribute towards increasing the concentration values found in the tissue. As has already been said the volume of the capillary blood in the brain only constitutes rather more than one per cent of the weight of the normal human brain. The amount of interstitial fluid is difficult to estimate. However, the histological examination of the brain showed, in both healthy and infected animals under the prescribed experimental conditions, that the Sp. was mainly localized at the tissue and thus revealed an affinity for certain elements, namely the ganglia cells; it is therefore probable that the raised concentration values depend for the most part on an increase of the Sp. content in the tissue and these tissue elements. This increase may of course be "secondary" to an increase in the Sp.-concentration in the interstitial fluid.

The above-mentioned change in the amount of blood in the capillaries and the quantity of interstitial fluid at infection is compensated, as previously stated, by certain forces, which thus also affect the increase of the Sp.-concentration in the tissue. The lymphatic system, which has great significance in this connection, is considered to have a lower capacity in the brain than in other organs. This fact should be able partly to explain the particularly marked increase in Sp.'s permeability to the brain in infected animals.

Clinical Section.

As the investigation of the clinical material shows, an increase of the permeability of sulfathiazole to the corpuscles is present in infected human beings. This is specially marked in infected cases with exanthema. Earlier investigators have found a causal connection between the permeability of sulfanilamide derivatives

to blood corpuscles and cerebrospinal fluid in the healthy subject. A similar connection has been shown by the author's experiments also to exist between the permeability of Sp. to corpuscles on the one hand and cerebrospinal fluid and tissue on the other, in healthy and infected animals alike. If these experiences are confirmed in an investigation on clinical material, then a determination of the permeability of sulfanilamide derivatives to corpuscles should attain a certain clinical value. It is possible that the degree of toxic lesion from the infection and the risk of toxic manifestations from the drug might be evaluated in this way—it might also be possible to draw prognostic conclusions and prevent serious complications of the drug.

The clinical and experimental investigations which have been carried out thus show: (cf. p. 28 author's problems)

1) The infection increases the permeability of Sp. to corpuscles, cerebrospinal fluid and tissue in animals.

2) The increase of permeability of Sp. in infected cases is particularly marked for the brain, where the drug shows an affinity for the protoplasm of the ganglia cells.

3) Thus, there is a chance that toxic symptoms may arise in infected individuals after a dose of Sp. that in healthy individuals does not set up toxic symptoms from the central nervous system.

4) Infection causes in man an increase of the permeability of sulfathiazole to corpuscles similar to the one previously described by me for Sp. in animals.

5) In those cases which showed toxic manifestations in the form of exanthema, the increase of the permeability for sulfathiazole in blood corpuscles is particularly marked.

SUMMARY.

The distribution of sulfapyridine in blood, cerebrospinal fluid and tissue after administration by different routes has been investigated experimentally in rabbits, both healthy specimens and specimens infected subarachnoidally and intracutaneously with pneumococci type 1. The distribution of sulfathiazole in corpuscles and plasma after peroral medication has been studied in a clinical material consisting of healthy subjects, cases of acute infections, and cases of exanthema.

The first chapter gives a review of the extensive literature on the subject, with special regard to certain toxic manifestations.

The second chapter introduces the problem. Here the author states the experimental and clinical observations of the effect of infection on the action of the drug.

Chapter 3 describes the material used, the arrangement of the experiments, and the methods followed.

Chapter 4 describes the course of infection in untreated animals, as also histological investigation of the brain during different phases of meningitis, and studies into the localization of sulfapyridine in the brain.

Chapter 5 discusses the time for taking samples after different methods of administering the drug. Samples have been taken half-an-hour after intravenous injection, one hour after intramuscular injection, and two hours after subcutaneous injection.

Chapters 6 and 7 contain an account of the distribution of sulfapyridine in blood, cerebrospinal fluid and tissue in healthy and infected animals respectively. An increase in the power of sulfapyridine to permeate to corpuscles, cerebrospinal fluid and tissue could be observed in the infected animals.

Chapter 8 discusses the effect of the size of the dose, and the effect of different methods of administration. It is shown that

the size of the dose has no significance, while the increase of permeation power for sulfapyridine in corpuscles, cerebrospinal fluid and tissue in the infected animals is most pronounced after intravenous medication.

Chapter 9 describes in a series of animals the distribution of sulfapyrdine in the organism of one and the same specimen before, during and after infection.

Chapter 10 collocates the degree of acetylation in healthy and infected animals respectively.

In Chapter 11 it is shown that a sex difference as regards the distribution of sulfapyridine is very probable though not significant. The female animals showed somewhat higher power of permeation to corpuscles and cerebrospinal fluid for sulfapyridine.

Chapter 12 describes dialysis experiments into the sulfapyridine's conditions in serum from healthy and infected animals. The serum from infected animals showed an increase of the dialyzable part of the drug as compared with that in serum from healthy animals.

Chapter 13 discusses the results obtained from the experimental investigations.

Chapter 14 collocates the investigations in the distribution of sulfathiazole between corpuscles and plasma in a clinical material. The cases of acute infection showed an increase in the power of sulfathiazole to permeate to corpuscles as compared with the same situation in healthy subjects. The increase was especially marked in the exanthema cases.

Chapter 15 discusses the observations made of the distribution of sulfanilamide derivatives in association with the derangements in the organism attendant on infection.

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